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Technical Transfer Report on a TNT Enzyluminescent Vapor Detection System

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Report Date
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Belvoir Research, Development and Engineering Center
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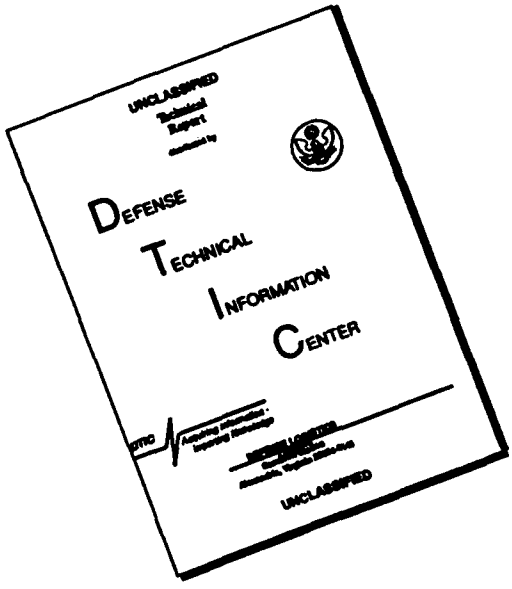

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This report describes the historical breadboard effort with enzyluminescent 2,4,6-trinitrotoluene (TNT) vapor detection system. The system comprises a sample train, vapor generator, and luminescence detector using a TNT reductase enzyme. This breadboard system is the culmination of a long-term effort evolving from several programs that examined the TNT enzyluminescent and bioluminescent response of marine bacteria, antibodies, and enzymes under BRDEC auspices.

This program was suspended by BRDEC because its slow rate-of-response (total more than 22-minutes sampling and detection time with a 0.25 part per trillion (ppt) TNT-in-air lower detection limit) and therefore cannot be used for mine detectors.

This report discusses an engineering design analysis that was performed for a new configuration of an enzyluminescent TNT vapor detection system that utilizes an integrated sample-reactor module to provide an estimated TNT rate-of-response of less than 60 seconds and a 0.0025 ppt minimum detectable concentration. This improvement can be of significant value in noncountermine applications. The most feasible and adaptable current application for the conceptual system is that of enclosure monitoring (building, room, etc.) for the presence of TNT vapors. The report describes system operation and use is examined in these applications.

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EXECUTIVE SUMMARY

The US Army Belvoir Research, Development and Engineering Center (BRDEC) has developed a 2,4,6-trinitrotoluene (TNT) explosives vapor detector in a breadboard configuration. Initially, the system's intended use was for antitank mine detection. However, evaluation of the system performance specifications showed that the sampling time required to attain a usable minimum detectable concentration (MDC) was **minutes**; it would have to be **seconds** for this application. For use as a mine detector, a highly sensitive and specific vapor detector is required in all configurations, i.e., a vehicle-mounted or man-portable system. These requirements arise because of the small explosives vapor mass available for sampling from a buried mine (burial depth reduces the amount of vapor above ground). A system time rate-of-response in the 100-ms range is required for a vehicle-mounted system. For most US Army mine detection applications, the vehicle, and thus the detector, speed is at least 3 mph. A system time rate-of-response of a few seconds is required for a man-portable unit. Enzymatic methods were originally chosen because of their inherent specificity and the bioluminescent methods because of their inherent sensitivity.

Although it appears that the sampling time can be shortened significantly with a radical design change, the program has been suspended. However, the Center believes the results of development are of significant value for other applications and is seeking to transfer this technology to other related programs.

The system comprises a sample train, vapor generator, and luminescence detector using a TNT reductase enzyme. It is the culmination of a long-term effort evolving from several programs that examined the TNT enzyluminescent and bioluminescent response of marine bacteria, antibodies, and enzymes.

The development program began with the isolation, production, and purification of a TNT-specific enzyme and proceeded to the design of a special-purpose sample train that preconcentrates the TNT air sample and absorbs it into a liquid water sample to interface with the detector. The enzymatic TNT detection scheme is based on a sensor and two indicator reactions as detailed in Section II. All reagents used in the reactions are contained in one solution, which emits light in proportion to the NADH concentration present. When TNT is present, the amount of light emitted is inversely proportional to the concentration of TNT in solution. In other words, a higher concentration of TNT would cause a decrease in the light signal.

Preliminary tests show that the TNT enzyme possesses excellent specificity. In vitro tests verified the enzyme response, sensitivity, and specificity to dissolved TNT molecules. Two reaction cells were developed for the detector. Both comprised a cylindrical packed bed through which sample and reagent streams flow and react to produce a transducible event. One cell contains the TNT enzyme immobilized on the packing surface, and the second cell contains co-immobilized bacterial luciferase and oxidoreductase reactants on its packing surface.

The system performs the TNT detection process intermittently over a 22-minute cycle with a 0.25 part-per-trillion (ppt) TNT-in-air lower detection limit. An approximate 3,000-L volume of air is processed over 20 minutes during which the sample train extracts TNT vapors with a 30 to 50 percent efficiency and dissolves them in a 3.5-mL liquid water volume that is subsequently pumped to the detector for analysis or transduction into a binary alarm signal.

An engineering analysis shows that significant performance gains can be obtained with a more fully engineered system. Several areas of the hardware are specifically identified. For example, changes to the reagent and sample pumps and the light detector photomultiplier tube assembly, together with increases in the sample flow rate and size of the reaction cells, will increase the signal-to-noise ratio of the system output and thus improve the system TNT MDC and/or time rate-of-response. More fully engineered reaction cells will improve reliability, maintainability, and consistency of performance. These improvements can be of significant value in other applications.

An examination of the system user requirements and corresponding performance specifications defined the system application limitations. The most feasible and adaptable current application for the system is that of enclosure monitoring (building, room, etc.) for the presence of TNT vapors. The system operation and use is examined in these applications. In addition, the Center's program defines concepts for a multiple explosives vapor detection and identification system using the developments obtained for the TNT vapor detection system. The basic items requiring development are the specific enzymes responsive to particular explosives species, e.g., nitroglycerine (NG), 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX), pentaerythritol tetranitrate (PETN), etc.

Although the Center is suspending the program at this time, it has

- Identified further developments required to improve the system,
- Examined the engineering status of each module,
- Defined concepts for other explosives vapor detection systems,
- Listed each system's commonalities,
- Performed an applications analysis, and
- Formulated a technology transfer scheme and process chart for the technology developed by the program.

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SECTION I
TECHNOLOGY TRANSFER PROCESS AND PROTOCOL

The Belvoir Research, Development and Engineering Center (BRDEC) has developed an enzyluminescent trinitrotoluene (TNT) vapor detection system in a breadboard configuration. Information on progress of the development over several years is contained in this report. All contracts and associated contract documentation and open literature publications on the contract work are included in Appendixes A, B, and C. A description of the hardware developed and its modular performance is presented together with conceptual designs of two additional vapor detection systems using enzyluminescent technology. Recommendations for design and component changes to the existing hardware are listed with corresponding estimates in performance gains.

The utility and limitations of the existing system are discussed in terms of its expected modular performance and the user requirements for three different detection scenarios. An applications analysis is used to assess the utility of the system for room monitoring applications and illustrates the use of the system.

All the conclusive information regarding the detection system development, design, and testing is included in this report. It can be of value as a guide for similar developments in general and enzyluminescent detection systems and devices in particular. The report layout allows access to the technology on either a broad or a focused basis.

Enzyluminescent technology transfer can be facilitated by illustrating its application to a vapor detection system development program. Figure 1 is an outline guide for system design for the program. It begins with defining the detection system and carries through the basic steps for developing fieldable hardware. The user of this technology can use all or part of the outline guide presented in Figure 1. Specific applications to each task are identified in terms of sections or appendixes of the report. In essence, all tasks as presented can be used independently and related to another task or subtask in any program or itself may constitute the end result.

The program layout will prove very useful and provides the reader with a utilitarian perspective of the information and its various uses.

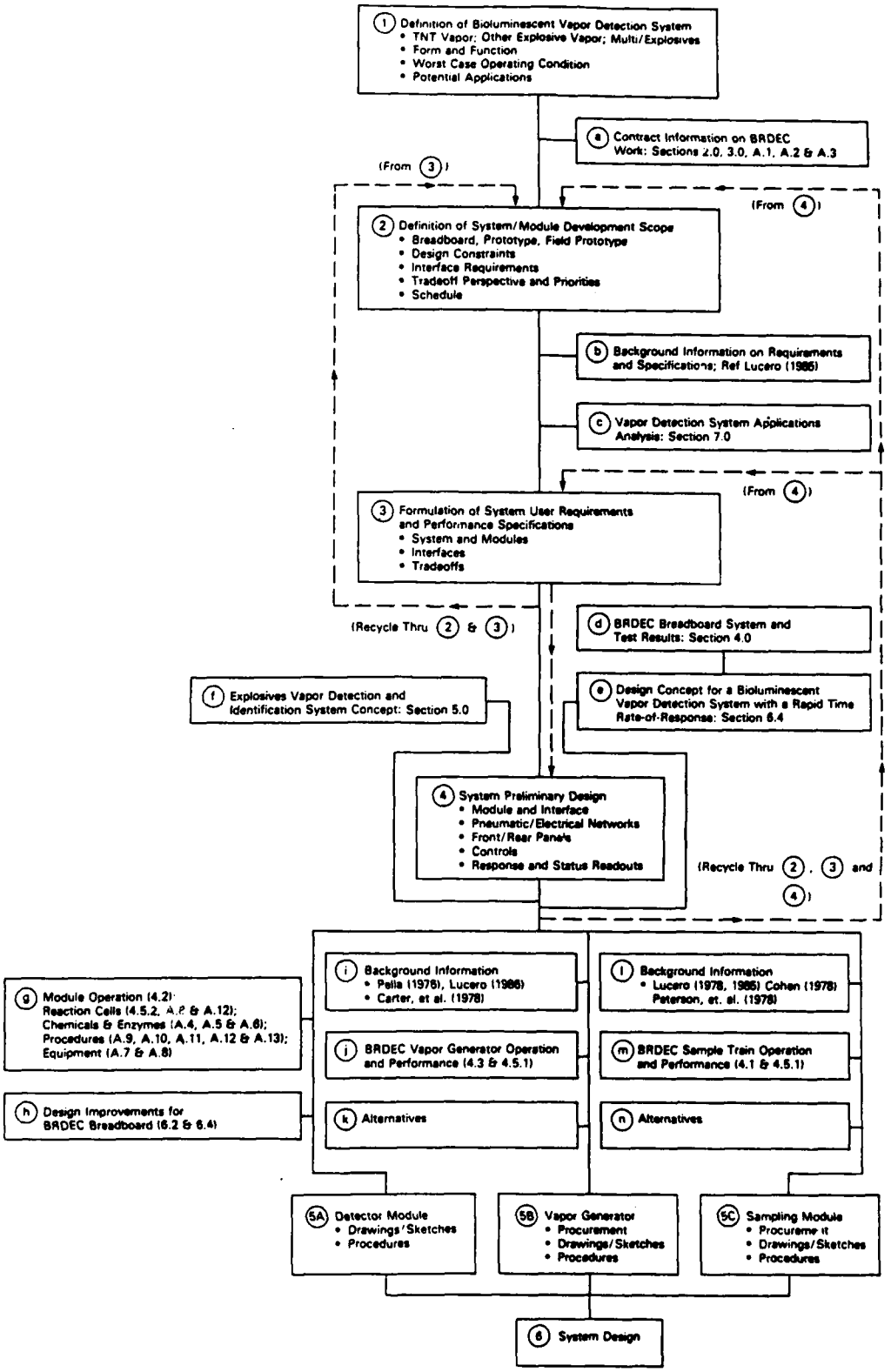

Figure 1. Program Task Layout

DEFINITION OF ENZYLUMINESCENT VAPOR DETECTION SYSTEM, TASK 1

It is essential to define the vapor detection system in terms of the following items.

Response Characteristics

Analyte response, minimum detectable concentration (MDC), specificity, maximum allowable interferent equivalent response, and maximum noise and drift;

Worst-Case Operating Conditions

Ambient temperature and humidity range, shock and vibration limits, maximum allowable service and maintenance frequency, and minimum allowable unattended operating time period;

Form and Function

Configuration (rack or bench mount), transportable, portable, automated operation, and automated alarms and consumables requiring periodic or continuous replacement; and

Potential Applications

This exercise, even in a preliminary form, can be very useful. It provides the basis for all successive tasks. The information obtained during this program is helpful in formulating a preliminary definition of a enzyluminescent vapor detection system for a wide range of different applications. This includes the following report items:

Section II:	Center Background Work
Section III:	Explosives Vapor Monitoring System
Appendix A:	Center Development Contract Chronology
Appendix B:	Contract Documentation Listing
Appendix C:	Contract Open Publications Listing

DEFINITION OF SYSTEM OR MODULE DEVELOPMENT SCOPE, TASK 2

The program goals and intended primary use of the hardware define the system or module development scope in task 2. It is defined in terms of:

- The general type of hardware to be developed: breadboard, prototype, field prototype.
- The primary design constraints: dimensions, performance, configuration, and operating conditions.
- The interface requirements: user, operator, site, and other systems or subsystems.
- A preliminary design and trade-off perspective as related to performance, configurational, schedule, and budget priorities.

The reader can be assisted in initiating this task by reviewing the following report items:

Reference 11: Lucero, 1985, background information on requirements and specifications
Section VII: Vapor Detection System Applications Analysis

FORMULATION OF SYSTEM USER REQUIREMENTS AND PERFORMANCE SPECIFICATIONS, TASK 3

A definitive description of the hardware at this stage is obtained with completion of task 3. The user requirements are formulated for the system only, and the corresponding performance specifications are formulated for the system and the primary modules. It is important to include the intramodular interface requirements and potential intramodular performance trade-offs. A general vapor detection system and its basic primary modules is presented in Section III—Explosives Monitoring System.

A listing of the parameters defining the basic system user requirements and corresponding system and modular performance specifications is provided in:

Reference 11: Lucero, 1985
Section VII: Vapor Detection System Applications Analysis

A review of Section VII and the specified reference can be of immense help in this task. The basic system and modular parameters are listed as well as suggested procedures and data required to quantify the specifications.

The results of tasks 1, 2, and 3 are dynamic and thus can change for a variety of design and programmatic reasons. In addition, the information developed with each is essential for the succeeding task and, many times, very helpful for the preceding task. Thus, it is common practice to repeat tasks 1, 2, and 3 with the information developed.

SYSTEM PRELIMINARY DESIGN, TASK 4

A preliminary design of the hardware is performed based on the information and constraints developed in the preceeding tasks. It includes design of the system and individual modules, interfaces, pneumatic and electrical networks, and front and rear panels. The design also defines the system controls and response and status readouts.

The following report items can help the reader in the progress of the system preliminary design:

Section IV: Enzyluminescent TNT Vapor Detection System
Section V: Enzyluminescent Explosives Vapor Detection and Identification System
Section VI: System Design Concept for a Rapid Time Rate-of-Response

Section IV provides design and performance information regarding the breadboard system designed under the BRDEC program. Section VI describes a radical design concept for reducing the system time rate-of-response. Depending on the system performance specifications defined by task 3, this design or another design of equally radical departure may be required. If a multi-explosives vapor detection system is a requirement, Section V describes an application of existing engineering and defines the system configuration to fulfill the system response requirements. It is noted that, in this event, an additional fundamental development stage is required to develop, produce, and purify specific enzymes for other explosives species.

MODULE DESIGNS, TASK 5

The vapor detection system, regardless of the results of tasks 2, 3, and 4, is best designed, assembled, and tested on a modular basis first, building to a system in the final stages.

Detector Module, Task 5a

Prior to and during the detector module design task, valuable information can be obtained from this report regarding the Center's current detector module design and design improvements examined by the author.

The information on the current design includes operational, procedural, and equipment. It is complete, allowing the user to review the information, test procedures, results, and equipment. The completeness permits the user to develop independent conclusions, extrapolate the data, and better plan for testing the new design. Sufficient information is present for complete replication of the design and tests of the the Center's breadboard:

Section IV:	Module operation
Appendixes D, E, and F:	Chemicals and enzymes
Appendixes G and H:	Equipment
Appendix I:	Procedures
Section IV, Appendixes H and I:	Reaction cells

A delineation of design improvements is also included in Section VI — Design improvements for the Center's breadboard.

These sections describe the design changes in relative detail and prescribe a procedure and a sequence for implementing the changes. The sequence prescribed must be followed to realize the improvements. It is important that drawings and sketches and special procedures be documented at the conclusion of the detector module design task 5a. With the documentation, the program proceeds to fabrication, assembly, and test.

Vapor Generator Design, Task 5b

The reader can use any vapor generator meeting the interface requirements of the sampling module. The Army program utilized the generator design described in Section IV. Documentation regarding this vapor generator is included in Appendixes B and K.

Sampling Module, Task 5c

The reader can use any sampling module meeting the interface requirements of the detector module. However, to obtain the MDC levels reached by the BRDEC system, the sampling module must provide a preconcentration factor equivalent to that obtained by the sampling module described in Section IV. Documentation regarding this sampling module is included in Appendixes B and J.

SECTION II
BRDEC PROGRAM BACKGROUND WORK

In 1970, the Center learned of the technique of using luminescent bacteria for trace-vapor detection and its potential to be developed into a compact man-portable system.

RPC Corporation of Segundo Beach, CA (no longer in business), demonstrated that TNT vapors heated to 100°C could be detected by bioluminescent bacteria. The bacteria was grown by RPC from marine environments of the California coast in liquid nutrient solutions and deposited on agar cartridges. To enhance the sensitivity, the bioluminescent bacteria was subjected to a variety of treatments, including exposure to chemicals and ultraviolet light to initiate mutagenesis, nutrient optimization, buffering to neutralize the acid formed by the bacteria, and special preparation to render the bacteria more nutritionally dependent on TNT. Sensitivity gains were minimal. The optimum MDC claimed was 30 parts per billion (ppb) (m/m). Specificity was also a problem: the bacteria reacted to many battlefield contaminants such as gasoline, motor oil, and smoke and to water vapor carried in the air. The bacteria also reacted to numerous other sources, in fact to anything that would alter its bioprocess.

While RPC conducted its research, Midwest Research Institute (MRI) of Kansas City, MO, was tasked to investigate the utility and sensitivity of bioluminescent bacteria suspensions in saline solutions. The sensitivity remained at approximately 30 ppb (m/m). There was no change or improvement in specificity.

Further investigations were performed by MRI on the effects of explosives vapors on in vitro bacterial bioluminescent (luciferase) reactions. The reaction was coupled with an alcohol dehydrogenase reaction, resulting in NADH regeneration that allowed light emission for long time periods approaching 23 hours. However, the in vitro bacterial bioluminescent reaction was affected by oxygen, which is a serious problem for a trace-gas detection system. The MDC from this approach was 10 ppb (m/m), with no specificity improvement for explosives vapors.

A 1973 observation that bacteria changed the TNT molecule chemically led to the idea of isolating an enzyme that effects such chemical changes from the bacterial cells. Because of the inherent specificity, Army personnel correctly reasoned that a TNT enzyme would provide the specificity for a TNT vapor detector. The concept for a TNT vapor detection system was based on coupling the enzymatic TNT reaction with a light-emitting biochemical indicator reaction. Thus, the original concept of using bioluminescence directly for TNT vapor detection was modified to use it as the indicator luminescent reaction in an enzymatic detection scheme. This concept comprised a primary reaction between TNT and the TNT enzyme, which itself was reacting with a co-enzyme, NADH. The TNT-TNT enzyme reaction competes with the NADH-TNT enzyme reaction. Subsequently, the reaction products undergo a second reaction, which produces a luminescense inversely proportional to the TNT concentration in the primary reaction. Thus, the original bioluminescent reaction used in TNT vapor detection is used as an indicator luminescence reaction in the enzymatic scheme.

Beckman Instruments Inc. (Carlsbad, CA), was contracted in 1976 to grow bacteria from soil in a TNT-containing media to induce TNT enzyme synthesis. The soil chosen had been subject to TNT pollution for many years. A large number of bacteria strains were grown and evaluated for their TNT enzyme producing capabilities, and a strain believed to be the best at that time was selected. Fermentation techniques were used to grow the cells of this strain, which served as raw material for enzyme isolation. Purification of the raw material by precipitation and chromatographic techniques produced a refined and stable TNT reductase enzyme, which can be lyophilized and stored for years. The enzyme contains a residual NADH oxidase activity, which catalyzes an unwanted side reaction.

The enzymatic TNT detection scheme is based on the sensor (1) and indicator (2) reactions:

$$TNT + 2\,NADH + 2\,H^+ \xrightarrow{\text{TNT reductase}} TNT_{(red)} + 2\,NAD + H^+ \qquad (1)$$

$$NADH + H^+ + FMN \xrightarrow{\text{Flavin reductase}} NAD^+ + FMNH_2 \qquad (2)$$

$$FMNH_2 + RCHO + O_2 \xrightarrow{\text{Luciferase}} FMN + RCOOH + H_2O + h\nu \qquad (3)$$

All the reagents involved in the reaction are contained in one solution, which emits light depending on the NADH concentration. With TNT present in solution, the reactions provide a decrease in the light signal. The enzymatic scheme and the isolated enzyme was used to demonstrate experimentally a TNT MDC in solution of 2×10^{-14} mole. Specificity was not examined by Beckman, but Army personnel reasoned that the TNT reductase enzyme would show some degree of response to 2,4-dinitrotoluene (DNT) and analogous compounds. This was not considered to be a drawback, because DNT might be more available than TNT for military explosives.

In 1979, the University of California, San Diego (UCSD), initiated research for TNT detection by enzyme immunoassay in which specificity is provided by antibodies. A bioluminescent immunoassay for TNT was developed that responded to 10^{-18} mole of TNT in solution, but it required several hours to complete. The Army abandoned the technique because it appeared more useful for forensics or pollution evaluation than mine detection.

The focus of research returned to the TNT reductase enzyme technique. Using the bacteria strain developed by Beckman, UCSD was able to increase the amount of enzyme obtained from the bacteria by a factor of 14 and significantly reduced the contaminating NADH oxidase activity from 15 percent of the TNT reductase, in crude extracts, to 0.1 percent in a partially purified enzyme.

The first steps in the breadboard development of a TNT vapor detection system began with the immobilization and co-immobilization of the enzymes described above. A model of a packed bed reaction cell (the flow cell) was assembled, and a TNT response was obtained. Concurrently, the Center contracted MRI to develop a gas/liquid interface and a gas-phase calibrator.

UCSD research continued, cloning the TNT reductase enzyme to obtain practical amounts for further research. The cloned enzyme showed slightly increased sensitivity over that produced by soil bacteria.

Specificity of the cloned enzyme was examined using ambient air samples from a variety of real-world situations. The first sample series showed favorable results and correlation between UCSD and MRI. Unfortunately, the second series was not as successful. The reason for the discrepancies obtained was not examined. More recently, the TNT reductase enzyme underwent a series of tests to determine whether it was indeed as specific as believed. Eight explosives were tested, including DNT, 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX), ethylene glycoldinitrate (EGDN), diethylineglycol dinitrate (DEGDN), pentaerythritol tetranitrate (PETN), Semtex, nitroglycerine (NG), and C-4. Only DNT responded with a relative response of 0.028. In addition, nitrofuran and nitropyradene, both nitroheterocyclic compounds, responded with a relative response near 0.028.

A TNT vapor detection system breadboard was assembled comprising a detector module, sample train, and calibrator. Its TNT vapor MDC was 0.01 ppt in 10 minutes. However, the MDC and time rate-of-response requirements for a viable mine detector are 0.001 ppt within seconds. Even with improvements in the signal-to-noise ratio, the enzymatic reaction time requirements seriously limit the TNT vapor detection system. It is not anticipated that the sampling time, using current designs, can be reduced below approximately 5 minutes.

The Army has concluded that the enzymatic TNT vapor detection system has been sufficiently explored, and it is not a viable candidate for a mine detector given the current technology state. However, it shows potential as a room air monitor or building air monitor. Potential development could lead to an explosives vapor detection and identification system as described in Section V. Because the Center does not have the Army's charter to develop room air monitors, funding has been withdrawn from the program. The authors anticipate that other Government organizations can benefit from this research and the equipment developed.

SECTION III
EXPLOSIVES VAPOR MONITORING SYSTEM

Vapor monitoring systems used to detect the presence of a clandestine explosives cache require ultra-low-level detection capabilities[11] (Lucero, 1985) as summarized in Table 1. An examination of the user requirements and the performance characteristics of the subject enzyluminescent TNT vapor detection system show that the utility of the system is restricted primarily to enclosure monitoring applications and suspect site predetonation examination.

The requirements most critically affecting the performance specifications of the system are the processing time to perform the detection and the false- and missed-alarm probabilities. Further examination of the detection requirements and consideration of the practical aspects of the detection procedures[11, 12] (Lucero, 1985; Lucero and Boncyk, 1986) suggest an MDC of 0.001 ppt and an alarm set point concentration level near 0.005 ppt.

Online gas stream monitoring systems operating at ultra-low-level detection limits, e.g., 1 ppt (v/v), require extractive analytical techniques with a special-purpose sample train and vapor-generating gas calibration device in support of the detector module. Figure 2 shows the basic modules and arrangement of a system. The sample train provides extensive sample processing[11, 12] (Lucero, 1985; Lucero and Boncyk, 1986) in the form of large sample preconcentration, and it effects a gas-to-liquid phase interface. A vapor-generating calibrator is used to periodically and quantitatively assess, by a vapor challenge, the system signal and zero

instabilities such that periodic system response readjustments can be made to null these instabilities. Frequent calibrations can reduce proportionally the system false-alarm probability.

A review of the MDC and other performance characteristics of explosives detector modules available commercially [24, 1, 19, 7] (Williams and Syverson, 1981; Aerospace, 1977; Proceedings, 1978; Lucero, 1985) and in development indicates that the sample train must efficiently transport explosives vapor molecules to the detector module, i.e., with a minimum sample loss, and produce massive explosives vapor preconcentration[11] (Lucero, 1985). These functions are performed by the special-purpose sample train used by the enzyluminescence detection system, thus permitting the detector module to attain a TNT MDC near 0.25 ppt (v/v) in air in a 22-minute detection cycle. An MDC near 0.001 ppt may be attained by improvements to the system liquid pumps and signal processing. In addition, it is estimated by engineering analysis that these improvements to the enzyluminescent system will permit operation near 0.0018-ppt alarm set point concentration levels with false-alarm and missed-alarm probabilities of 0.27 percent over 8-hour operating periods with a single 30-minute calibration episode.

Table 1. Explosives Vapor Detection System User Requirements and Performance Specifications [4, 5]

Application/Requirement	Processing Item/Personnel Examination @ Processing Station	Searching Area Search and Examination	Monitoring Enclosure Monitoring
Maximum Missed-Alarm Probability	$<5 \times 10^{-4}$	$<5 \times 10^{-4}$	$<5 \times 10^{-4}$
Maximum False-Alarm Probability	$<5 \times 10^{-4}$	<0.05	$<5 \times 1^{-4}$
Processing/Examination Rate	15 (Item-People/Min.)	$28 m^3$/Min. (1000 Ft^3/Min.)	Continuous
Minimum Detectable Concentration @ Sample Train Inlet, ppt	1	1	0.001
Maximum Allowable Alarm Set Point Concentration, ppt	5	5	0.005
Maximunm Allowable False-Alarm Probability @ 5 ppt	$<10^{-4}$	<0.004	10^{-4}
Maximum Allowable Missed Alarm Probability @ 10 ppt	$<10^{-4}$	<0.004	$<10^{-4}$
Maximum False-Alarm Probability with Interferent @ 1ppt	2.3×10^{-3}	<0.06	2.3×10^{-3}
Maximum Missed-Alarm Probability with Interferent @ 1 ppt	2.3×10^{-3}	<0.06	2.3×10^{-3}

SECTION IV
ENZYLUMINESCENT TNT VAPOR DETECTION SYSTEM

An enzyluminescent TNT vapor detection system utilizing a previously isolated enzyme that showed significant TNT reductase activity was developed for special-purpose applications, e.g., to detect the presence of a clandestine explosives cache in enclosures such as buildings or rooms. The development ended at the breadboard stage[2] (Bryant and Martin, 1987).

The system is a continuous wet-chemical detector module interfaced to an intermittent gas-phase sampler/preconcentrator and comprises sample train, detector, and calibrator modules. It responds solely to TNT vapor in air at sub-ppt (v/v) concentration levels. System operation is based primarily on two sequential liquid-phase biochemical reactions, between TNT and special reagents, promoted by the catalytic action of the TNT enzyme developed for BRDEC for this purpose[5, 3] (Egghart, 1982; DeLuca and Vellom, 1982). The final reaction product, the transducible event, is a luminescence, the intensity of which is inversely proportional to the TNT concentration in air.

SAMPLE TRAIN

Operation of the enzyluminescence TNT vapor detection system is based on the action of the individual modules of Figure 2 on the sample airstream. The overall process is described by tracing the flow of TNT molecules through the system.

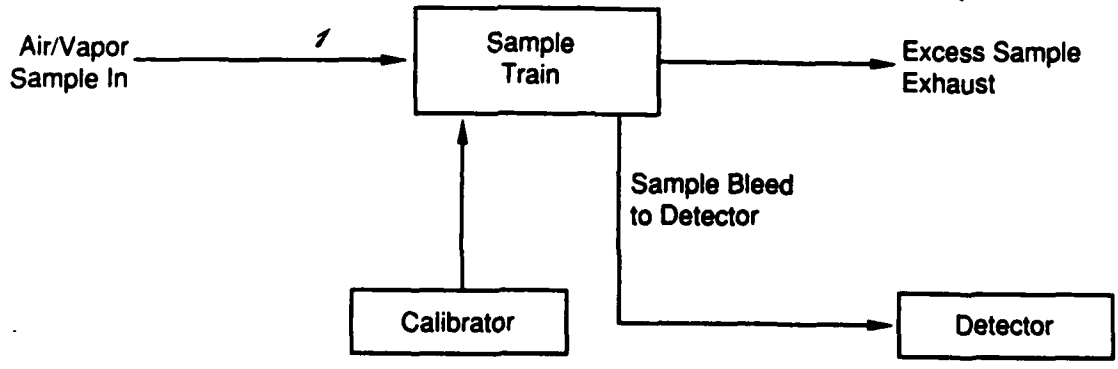

Figure 2. Vapor Detection System

The sample train performs the gas-phase sampling, preconcentration, and detector module interface functions. Its operation is based on the action of a liquid water gas extractor (MRI, 1985), which obtains a 429,000 preconcentration factor after processing a 150-L/minute sample airstream for 20 minutes with a 50 percent TNT extraction efficiency. At these operating conditions, the preconcentrator produces a 3.5-mL liquid water sample.

System operation is in an automated batch mode over a specific time period. A sample airstream passes continuously through the sample train. Simultaneously, a recirculating liquid water stream flowing in the sample train extracts a fraction of the TNT molecules from the airstream and stores and accumulates them in liquid solution. At the end of the sampling period, the liquid solution is removed from the sample train and is transported to the detector for analysis[12] (Lucero and Boncyk, 1986). In effect, a gas-phase sample is transformed into a liquid sample preconcentrated in TNT to interface with the liquid-phase detector module.

A spinning liquid water film is the basis of the sample train used by the enzyluminescent TNT vapor detection system. It produces a preconcentration factor described below:

$$P_f = C_o/C_i \tag{4}$$

where

P_f = preconcentration factor, dimensionless;
C_o = explosives vapor concentration out of the preconcentrator, ppt; and
C_i = explosives vapor concentration into the preconcentrator, ppt.

and

$$P_f = \eta_g Q_g(T_s)/V_1 \tag{5}$$

where

Q_g = gas flow rate, mL/min;
T_s = gas sampling time increment, min;
V_1 = liquid sample volume obtained from the preconcentrator, mL; and
η_g = TNT mass exchange or collection efficiency, dimensionless.

Figure 3 illustrates the sample train schematically. The functional center of the system is the sample processor. All other subsystems and modules act to support its operation. Sample air enters the sample processor where it immediately encounters the spinning liquid water film, and the TNT vapor molecule collection process begins. Upon emerging from the sample processor, the air sample stream flows through the liquid water trap to the sample gas flow rate control network and is vented from the system. Liquid water is supplied to the sample processor from a reservoir through the liquid pump. At the end of the air sampling process, the liquid water remaining in the sample processor is transported by the liquid sample pump to the detector.

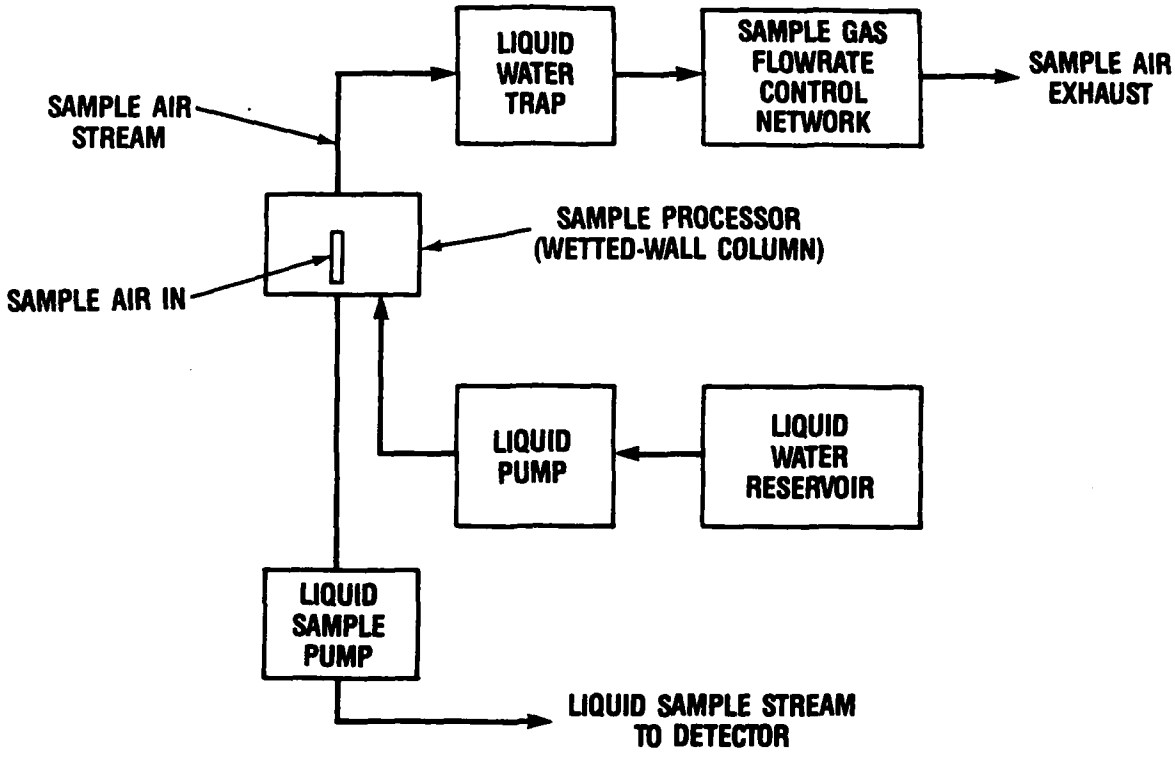

Figure 3. Sample Train Functional Schematic

The sample train TNT collection or extraction efficiency is[12] (Lucero and Boncyk, 1986)

$$\eta_g = 1 - \exp\left[-4\, k_m\, S\, D_t / V_g\, (D_t^2 - D_i^2)\right] \qquad (6)$$

where

S = column length, cm;
k_m = TNT mass transfer coefficient of the gas boundary layer, cm/s;
V_g = average gas velocity in the column, cm/s;
D_t = column diameter, cm; and
D_i = semi-stagnant air core diameter, cm.

The airflow within the sample processor moves in a circular helical fashion along the column circumference, as described below. Because of this airflow pattern, a core of semi-stagnant air or air moving upward at a relatively low velocity exists within the sample processor. Thus, the larger fraction of TNT transport is from the higher velocity air adjacent to the liquid film. D_i in equation (6) reflects this condition.

The sample processor is configured in the form of an upright circular cylinder with a small length-to-diameter (L/D) ratio (L/D = 1.5) as depicted by Figure 4: L = 7.6 cm, D = 5.1 cm. Its ends are penetrated by access ports to introduce and remove liquid water and the sample airstream. A sampling cycle consists of three steps: liquid water injection, air sampling, and liquid water sample removal. Initially, the sample processor is charged with 3 mL of liquid water by the liquid pump. The vacuum blower is turned on, and air is sampled at 150 mL/min for a set time period. Sample gas enters through the tangential slit in the sample processor.

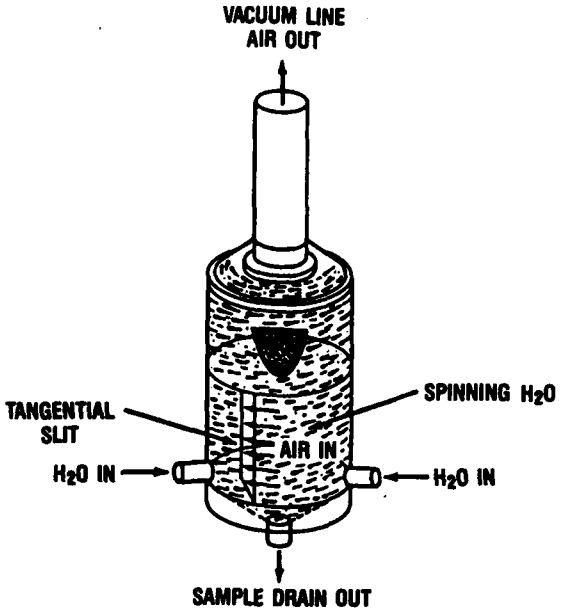

Figure 4. Sample Processor

Within the sample processor, the aerodynamic conditions continue to promote the spinning liquid in an upward circumferential direction, i.e., helical. Assuming that the bulk of the air sample also flows in an upward circumferential fashion and in a 1.3-cm annular gap, the air velocity is 520 cm/s and the Reynolds number is 6720. The TNT mass transfer coefficient (k_m) of equation (6) at these conditions and for a TNT molecular diffusion coefficient of 0.043 cm^2/s is 0.98 cm/s[12] (Lucero and Boncyk, 1986). It is estimated by equation (6) that the TNT sample processor collection efficiency is 37 percent at the operating condition described above. Experiments were performed with the sample processor to verify this estimate.

At the end of the air sampling period, the vacuum in the sample gas flow rate control network is shut down, the airflow ceases, and the liquid water film collapses to the bottom of the cylinder. Subsequently, the liquid water containing the extracted TNT is transported by the action of the liquid sample pump to the detector for analysis.

DETECTOR MODULE

Figure 5 is a schematic network of the detector module. It comprises the hydrodynamic network, reagent reservoirs, liquid sample stream, TNT_{ase} and luminescence reaction cells, and light detector.

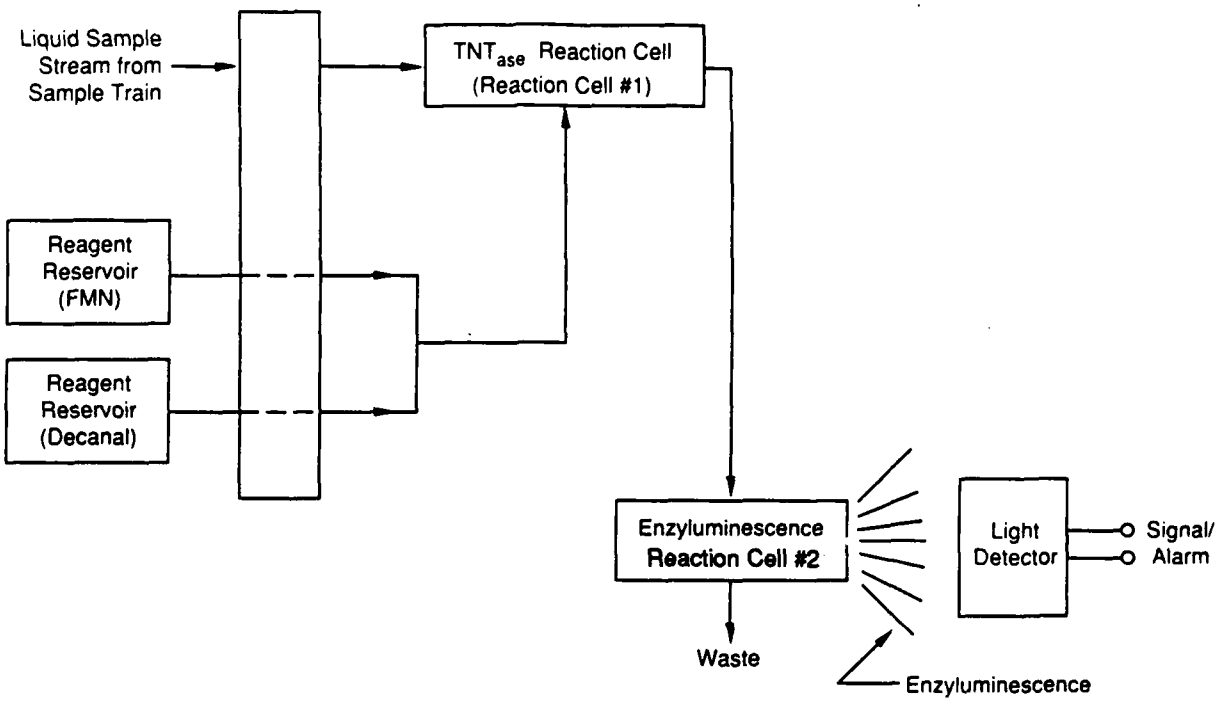

Figure 5. Enzyluminescent TNT Detector Module

In the detector module, the sample liquid stream converges continuously with reagent liquids at a chemical reactor, the TNT_{ase} reaction cell. The TNT and reagent reaction is catalyzed by a TNT enzyme immobilized on the surface of the reactor bed. Subsequently, the reaction product stream flows to a second chemical reactor, the luminescence reaction cell, where a second surface reaction occurs, producing luminescence as one of the reaction products. The luminescence intensity is inversely proportional to the concentration of TNT in air[4, 5] (Egghart, 1978, 1982).

The first reactor, the TNT_{ase} cell is supplied with separate decanal and FMN reagent[22, 8] (Vellom et al., 1984; Kricka and Vellom, 1986) streams and the liquid sample stream at 0.037 ml/min each. Two primary reactions can occur: one reaction without TNT present and the second with TNT present. The reagents react as follows:

$$NADH + H^+ + FMN \xrightarrow{\text{Flavin reductase}} NAD^+ + FMNH_2 \qquad (7)$$

and

$$TNT + 2\,NADH + 2\,H^+ \xrightarrow{\text{TNT reductase}} TNT_{(red)} + 2\,NAD + H^+ \qquad (8)$$

Reaction (8) occurring in the TNT_{ase} cell is the reduction of TNT promoted by the catalytic action of the TNT enzyme, the TNT reductase.

Reaction (8) competes directly with reaction (7) for NADH, resulting in a proportional decrease in the amount of $FMNH_2$ and, subsequently, a decrease in the intensity of luminescence produced in the luminescence reaction cell as described below by reaction (9). The TNT concentration in the sample liquid stream is proportional to the decrease of luminescent intensity as measured by the light detector (photomultiplier tube) of Figure 5.

The reaction products and the remnants of the three liquid streams are transported in a single stream to the second reactor, the luminescence reaction cell, where the following reaction occurs:

$$FMNH_2 + RCHO + O_2 \xrightarrow{\text{Luciferase}} FMN + RCOOH + H_2O + h\nu \qquad (9)$$

Reaction (9) is catalyzed by an oxidoreductase and by bacterial luciferase[3, 9] (DeLuca and Vellom, 1979, 1982; Kricka et al., 1983). It utilizes the FMNH2 in the presence of a long chain aldehyde and oxygen to produce the luminescence (hv). Both catalysts are co-immobilized on the reaction cell matrixes[23] (Weinhauser et al., 1982).

Practical implementation of this detector module is based on the fabrication of the TNT_{ase} reaction and luminescence cells. It is accomplished by the design of a heterogeneous reactor bed onto which the TNT_{ase} enzyme is efficiently immobilized in the TNT_{ase} reaction cell, while the bacterial luciferase and oxidoreductase enzymes are co-immobilized in the luminescence reaction cell. A microbore guard column packed with activated sepharose 4B matrix comprises the TNT_{ase} reaction cell. It is a CTFE rod with a drilled hole 1 mm in diameter and 2 cm long. The luminescence cell is a 10-μL Drummond Microcap column packed with activated sepharose 4B matrix[23, 3] (Weinhauser et al., 1982; DeLuca and Vellom, 1982). Figure 6 depicts the luminescence reaction cell assembly.

Detailed descriptions of the reaction cell assemblies, the reagents, formulations, immobilization procedures, etc., are in the related appendices.

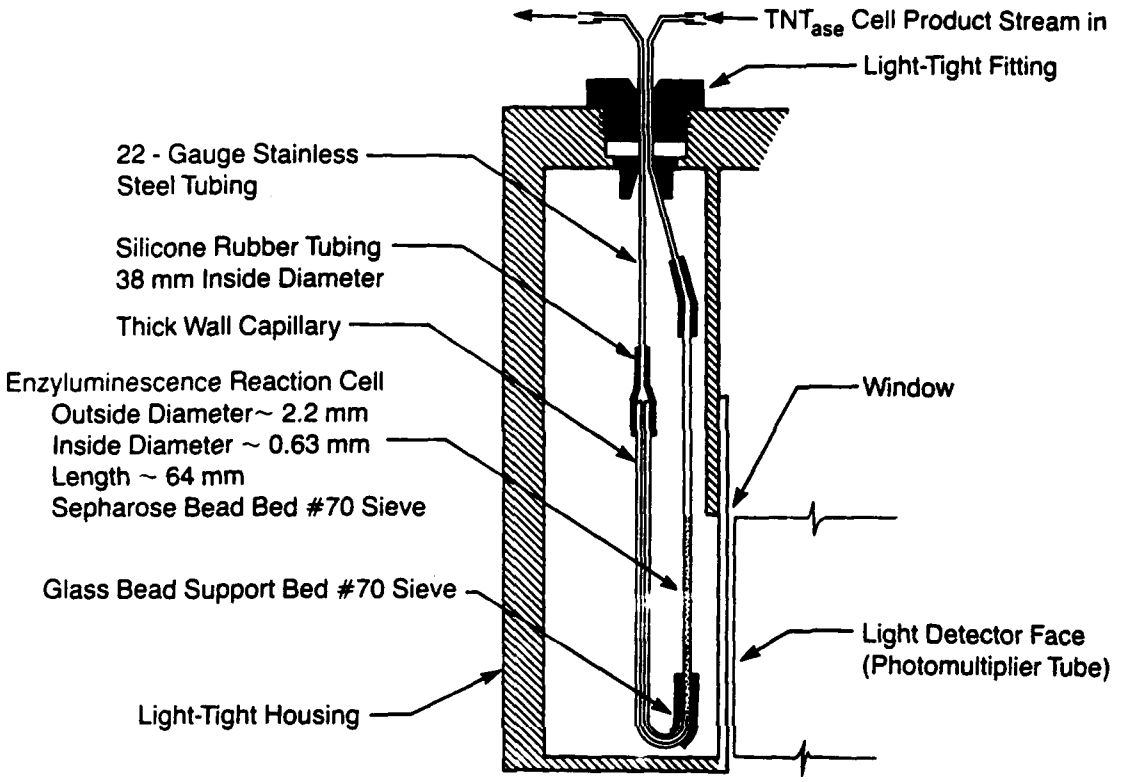

Figure 6. Enzyluminescent Reaction Cell [11]

VAPOR GENERATOR

A system TNT challenge is performed periodically by a calibrator, which is a device that dynamically generates a calibration gas, i.e., TNT in air, at known and controlled TNT concentration levels near the detection system alarm set point concentration. Figure 7 is a schematic depiction of the calibrator. Calibration gas is produced in a thermally controlled, packed-bed column containing a pure TNT coating on the bed particles. Inert carrier gas (in this case, nitrogen) is passed continuously through the column and is saturated with TNT vapor at the equilibrium concentration level established by the column temperature. The carrier gas and TNT mixture is transported continuously and is injected into the sample train. At the end of the calibration cycle, the system signal is adjusted to read the TNT concentration level produced by the calibrator, and the system returns to the monitoring mode.

The net TNT concentration after injection of the calibration gas into the sample train in this system is 0.1 ppt (v/v)[15, 17] (Pella, 1976; MRI, 1988).

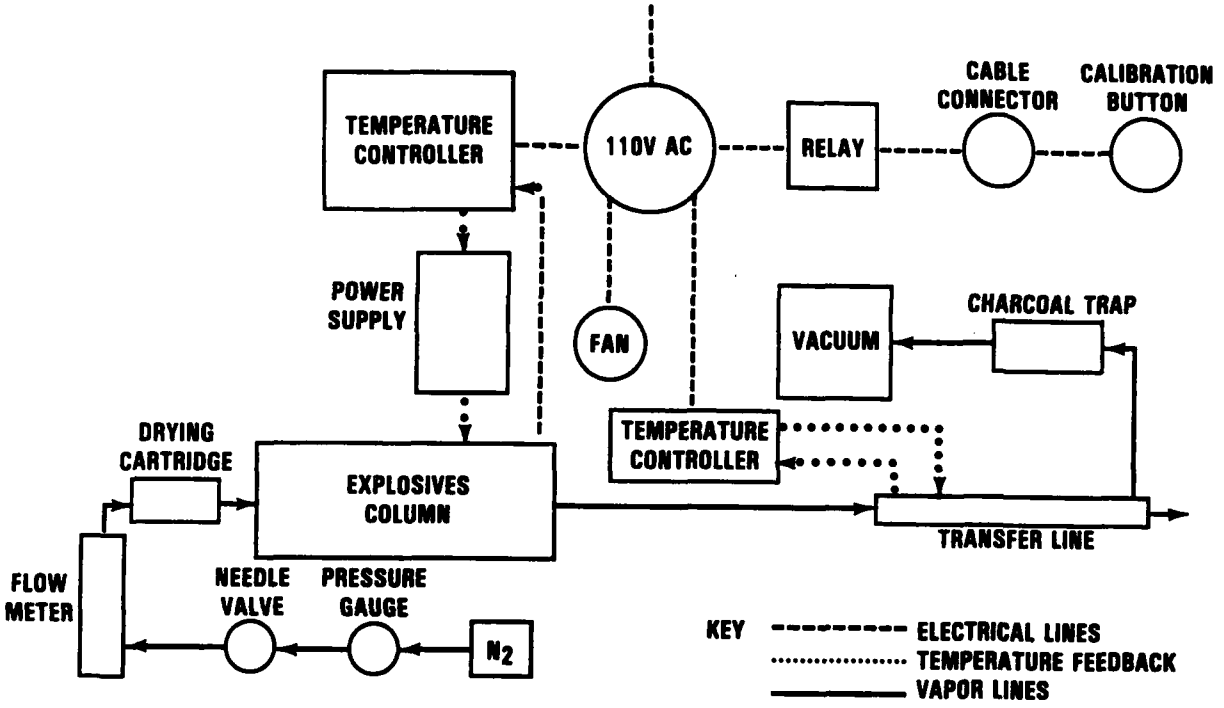

Figure 7. Caliber Schematic

DETECTOR MODULE EXPERIMENTAL EVALUATION

Detector TNT response data were obtained by liquid sample injections to the detector module. Each injection comprised a 100-μL liquid sample flowing at 37 μL/min for 2.7 minutes. The sample size was sufficient to obtain a response plateau and to obtain response, lag, rise, and fall time data. The TNT samples ranged in concentration from 1×10^{-8} to 2×10^{-8} mole, which corresponds to 2.1- to 4.2-ppt (v/v) TNT concentrations in air, respectively, for a 5-minute sampling period. Zero liquid sample was injected in an identical fashion.

The detector module was interfaced to an LKB Luminometer Model 1250 and a five-channel Ismatec IPN Model 7618-40 peristaltic pump. A continuous detector output reading was obtained on a strip chart recorder as illustrated by Figure 8. All data to establish the detector module performance specifications were obtained from the recording of detector module span and zero responses. These include lag time (t_L), rise time (t_R), fall time (t_F), span response (R_1, R_2, etc.), and noise. Span drift (percent full scale response/min), zero drift (percent full scale response/min), MDC (ppt), and linearity (percent full scale), were calculated from these data.

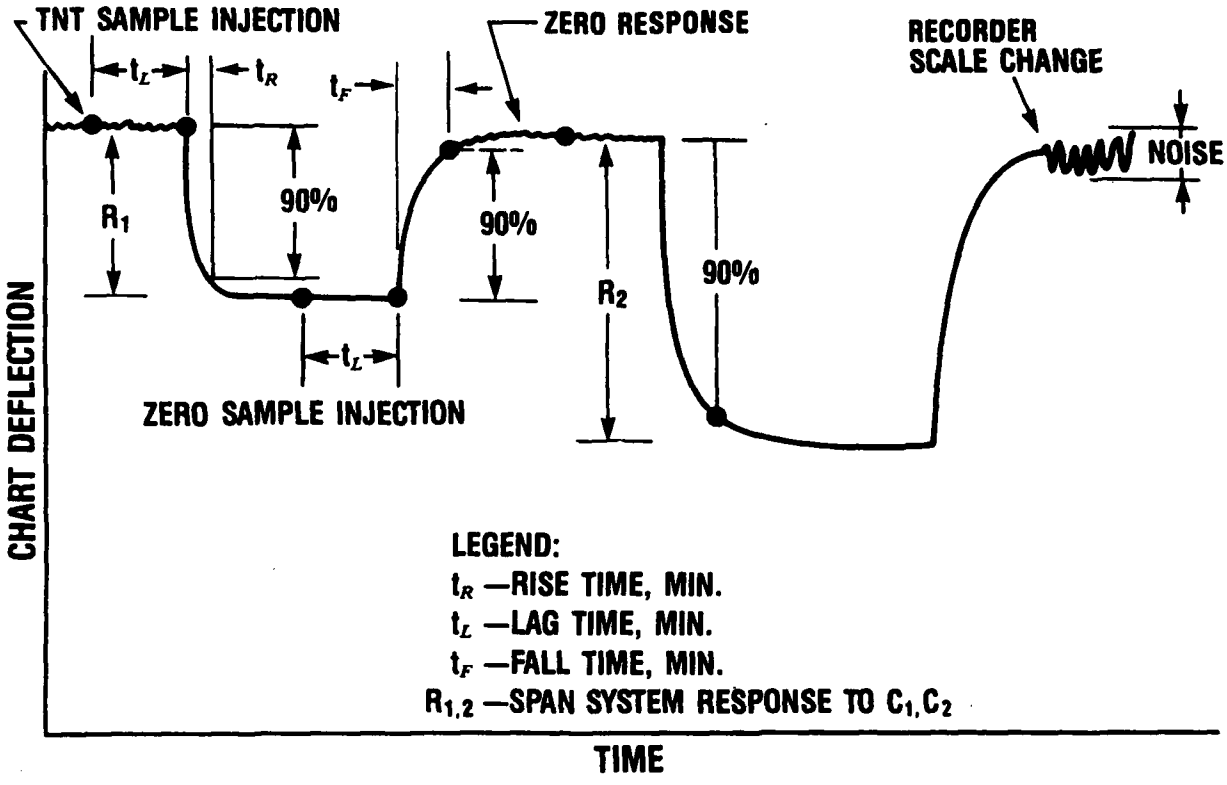

Figure 8. TNT Enzyluminescence Detection System Response

EXPERIMENTAL RESULTS

The performance of the modules depicted in Figure 2 (page11) were evaluated independently.

Sample Train and Calibrator

The sample train and calibrator performance specifications are listed in Table 2[15] (MRI, 1988). These correspond only to the pneumatic, hydrodynamic, and thermal conditions listed. The preconcentration factors listed apply only to a 3.5-mL sample volume. A large reduction in TNT sample collection efficiency was noted at sample volumes below 3.5 mL, which was imputed to incomplete coverage of the wetted-wall column gas extractor module surface with liquid water.

Table 2. Operating Specifications[1]

System operating parameters are selected to obtain a system response to TNT-in-air samples. To generate TNT concentration samples over 0.01 to 1.0 ppt, the output TNT concentration of the calibrator must be varied by a combination of changes to the calibrator output flow rate and TNT column temperature. Specifications were obtained from independent module tests.

MODULE	OPERATING PARAMETER VALUES
Sample Train	
Sample flow rate (L/min)	150
Liquid volume discharge (mL)	3.5
Sampling time (min)	5, 10, 15, 20
TNT input concentration (ppt)[2]	0.014 to 0.28
Preconcentration:	
(1) 5-min sample time	107,000
(2) 20-min sample time	429,000
Calibrator	
Column temperature (°C)	5 to 20
TNT output concentration (ppb)	0.7 to 3.5
Carrier gas flow rate (mL/min)	
3 to 12	
Detector Module[3]	
Decanal flow rate (mL/min)	0.037
FMN flow rate (mL/min)	0.037
Sample flow rate (mL/min)	0.037
Noise equivalent TNT concentration[4] in liquid solution (nM/L)	2.4
Minimum detectable concentration (ppt):	
(1) 5-min sample time	1
(2) 20-min sample time	0.25
Average time rate-of-response (s):	
(1) rise time	53
(2) fall time	53
(3) lag time	50
(4) total time	103
Linearity (percent)	Not determined
Maximum span drift (percent full scale/h)	<+ 6%
Maximum zero drift (percent full scale/h)	<+ 6%
Interferent equivalent response (ppt)[5]	No determination

1. D.P. Lucero, J. Testing and Evaluation 13, 1985, p. 222.
2. Resultant TNT input concentration after mixing calibrator output gas flow (3 to 12 mL/min) and sample train sample flow (150 L/min).
3. Federal Register 40, 33, 1975, pp. 7053-7057.
4. Noise equivalent concentration of TNT in air has no direct meaning because MDC is determined by preconcentration. Noise reduction is related only to liquid detector module.
5. Nitroheterocyclic compounds showed a 0.028 relative response per Michaelis-Menton measurements.

Detector Module

The raw data obtained were reduced from the form depicted in Figure 8 to the detector module TNT response and the operating specifications of Table 2. These data show that a 0.25-ppt TNT concentration level in air is detectable, i.e., MDC = 0.25 ppt, after a 20-minute sample time by the sample train. This means that the specifications of the modules comprising the system are adequate for the explosives vapor monitoring applications described earlier. The total time required by the detector is less than 105 seconds.

Experimental work to determine the detector zero and span instabilities was planned, but the program was halted before these data were obtained. Zero and span drift data were to be obtained for freshly prepared equilibrated reaction cells. Approximately 1 week is required for the reaction cells to stabilize after preparation, and additional time is required to check out the cells functionally prior to the zero and span drift tests. The program was near the end of its funding, and more essential tasks were given priority. Preliminary results show that maximum zero drift is less than ± 6 percent full scale per hour (full scale = 0 to 1 ppt). Nevertheless, the span and zero instabilities obtained to date show that additional work is required to stabilize both reactions (7) and (9) or reaction (9) alone. It is important to obtain low zero and span drift because they significantly affect the system false-alarm probability[11] (Lucero, 1985).

Some of the instability is due to the action of freshly prepared reaction cells. Freshly prepared cells typically exhibit a steady increase in the luminescent output of the luminescence for a period lasting approximately 1 week. Thereafter, the luminescence is stabilized, providing a proportionally stable zero. This suggests that manufacturing techniques can be developed to assemble more stable and more predictable TNT_{ase} and luminescence reaction cells.

Interferent Response

A quantitative indication of the TNTase specificity to TNT was obtained by subjecting a variety of explosives and possible interferent species to a prescreening process by the methods of the Michaelis-Menton kinetic assessment[21] (Stryer, 1988). This method provides a valid assessment for zero relative response measurements, i.e., a zero relative response measurement with the Michaelis-Menton method surely means a zero relative response of the TNT vapor detection system. A greater than zero response, however, usually requires further quantitative verification obtained from the detection system response.

A Cary Spectrophotometer model was used to measure the relative responses of various explosives and possible interferents to that response obtained from the TNT exposure to the TNT enzyme. The reference cell contained buffer (50 mM acetone, 50 mM MES, 100 mM TRIS at pH = 7.0, total volume of 990 µL) plus 10 µL of the TNT enzyme. The sample cell contained buffer, 10 µL TNT enzyme, 10 µL 30 mM NADH, and 10 µL of the explosive or interferent.

PETN, RDX, EGDN, NG, DEGDN, cyclotetramethylene tetranitramine (HMX), and Semtex showed zero relative response to TNT. DNT, nitrofuran, and nitropyradine had a 0.028 relative response to TNT. The relative response measurements showed an adequate specificity to TNT, thus excluding a response to other types of explosives. A small but measurable relative response to nitroheterocyclic compounds appeared. Further work is required to determine the interferent TNT equivalent response of these compounds.

Extensive interferent response tests were performed with atmospheric samples[15] (MRI, 1988). The results were inconclusive. Laboratory samples of pure water, sample train sample blanks, and sample train TNT samples showed reasonable correlation between a standard gas chromatography electron capture detector and the enzyluminescent techniques. However, atmospheric samples produced by the sample train over 10- to 30-minute sampling periods were inconclusive because of the lack of correlative measurements. Twenty-six background atmospheric liquid samples were obtained, eight of which showed a positive response. The conclusions regarding the positive TNT were not verified for TNT content by a reference technique. This was a serious omission that rendered results inconclusive.

SECTION V
ENZYLUMINESCENT EXPLOSIVES VAPOR DETECTION AND IDENTIFICATION SYSTEM

Based on the breadboard TNT vapor detection system described in Section IV, an identical design approach can be used to detect and identify vapors from several different explosives. The approach uses specific enzymes responsive to specific explosives to perform the enzyluminescent transduction. It is a viable approach in view of the enzyme preliminary specificity test results of Section IV, i.e., the enzyme displaying a 0.028 relative response to DNT and a zero relative response to PETN, RDX, EGDN, NG, DEGDN, HMX, and Semtex.

With a system utilizing enzymes specifically responsive to other explosives, such as NG, EGDN, RDX, HMX, and PETN, to catalyze similar reactions between the reagents and the explosives vapor molecules listed, the explosives detection and identification can be performed.

The author has examined the feasibility and utility of an explosives vapor detection and identification system using enzymatic enzyluminescent techniques. It appears that the system can be of significant utility in pre- and post-bomb detonation inspection scenarios[13] (McBride, 1989). A system exhibiting performance specifications similar to those of Table 2 (page 20) for the enzyluminescent TNT vapor detection system can be made adequate with design changes

described in Section VI for monitoring enclosures (as summarized by Table 1 (page 10)) for the explosives most favored by terrorists or underworld bombers.

Conceptually, the multi-explosives detection and identification system comprises the modules of Figure 2 (page 11) and the same sample train and calibrator used by the enzyluminescent detector described in Section IV. A calibrator or vapor generator carrying a multitude of explosives sources will be required, otherwise the system is identical to the TNT vapor generator[17, 15] (Pella, 1976; MRI, 1988).

An array of enzymatic reaction cells are required by the detector module, each specific to a corresponding type of explosive. For example, one configuration of the detector module is shown by Figure 9. Four reaction cells are arrayed in parallel hydrodynamically: $EXPLO_{ase}$, TNT_{ase}, $PETN_{ase}$, and RDX_{ase}. Each reaction cell of Figure 9 is identical to the TNTase reaction cells described earlier and differs only in that each is provided with the corresponding immobilized enzyme responsive to individual types of explosives. The $EXPLO_{ase}$ reaction cell carries all the enzymes co-immobilized on the substrate.

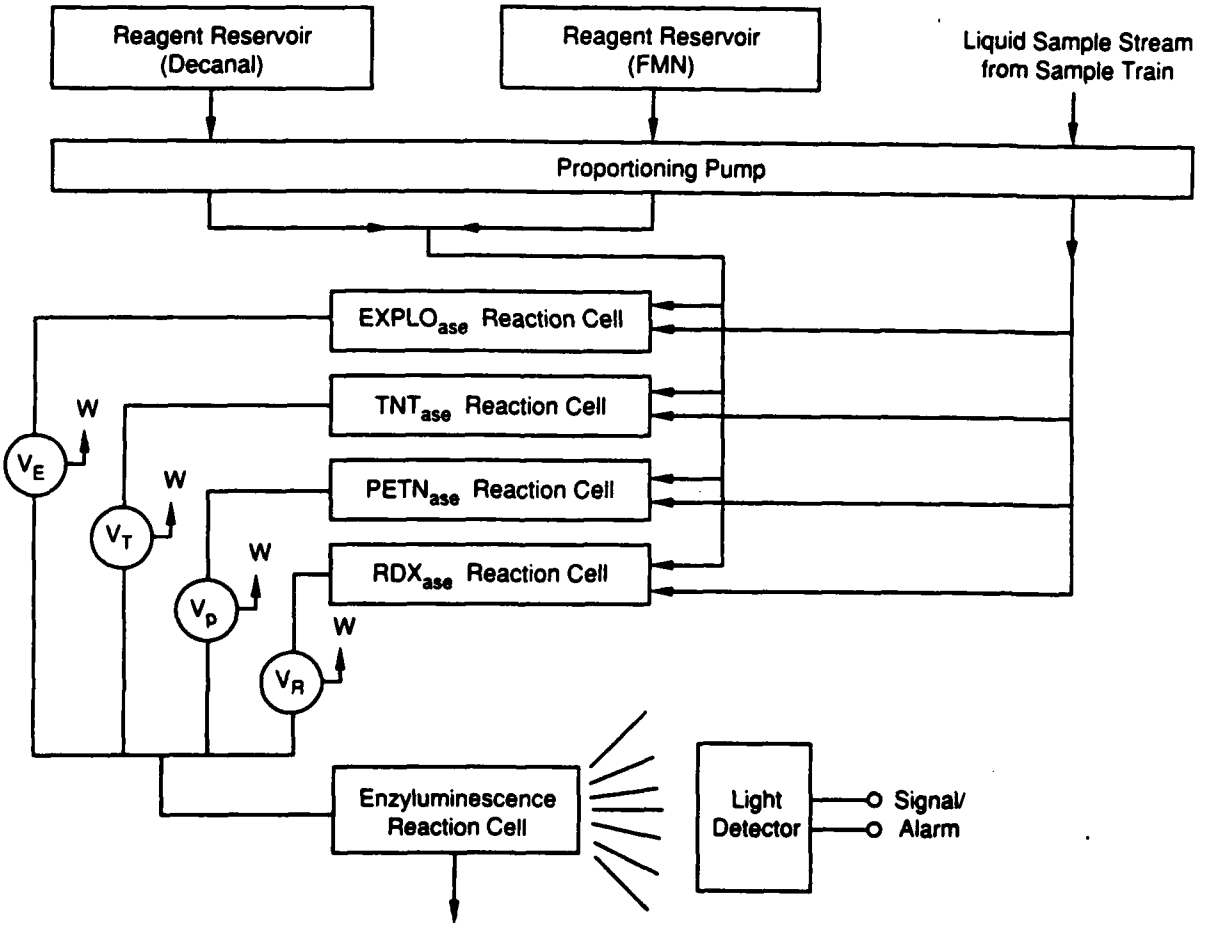

Figure 9. Enzyluminescent Explosives Vapor Detection and Identification Module

Reagent and sample streams flow continuously and converge at the inlet of each reaction cell. Each stream thereafter flows through its corresponding reaction cell and 3-way valve (V_E, V_T, V_P, and V_R). At this juncture, the resulting streams are diverted by each valve to waste (W) or to the enzyluminescence reaction cell.

For the no-general-alarm condition, i.e., TNT, PETN, and/or RDX vapors are absent in the sample or are present at concentration levels below the MDC of the system, the liquid sample and reagent streams flow continuously through the network of Figure 9. The stream emanating from the $EXPLO_{ase}$ reaction cell is diverted by V_E to the luminescence reaction cell and ultimately to waste. Concurrently, the liquid streams emanating from the TNT_{ase}, $PETN_{ase}$, and RDX_{ase} reaction cells are diverted to waste by V_T, V_P, and V_R, respectively.

In the event of a general alarm, the module 3-way valves are programmed into a sequential diversion of each reaction cell stream from waste to the luminescence reaction cell. During the entire sequential stream diversion process, V_E diverts the $EXPLO_{ase}$ stream to waste. An identification of the explosives vapor species is obtained by the stream that initiates a specific alarm, i.e., specific to the stream flowing through the luminescence cell at the time of the alarm.

The system is not limited to the three explosives species shown in Figure 9. Additional reaction cells may be added to the network as is deemed practical.

For the four stream networks depicted in Figure 9, the total sample flow rate is approximately 0.148 mL/minute or 0.037 mL/minute per stream, and the total reagent flow rate is 0.296 mL/minute or 0.074 mL/minute per stream. On the basis of the enzyluminescent TNT vapor detection system, the time required to perform the entire sequence is approximately 515 seconds (103 seconds per stream). An additional 103 seconds is required to clear the lines at the start of each sequence. A time increment of 103 seconds is added to the sequence for each additional stream added. The minimum total volume of liquid sample required per cycle is 1.27 mL, and the total processing time is 8.58 minutes.

SECTION VI
ENGINEERING ANALYSIS

The author performed an engineering analysis of the detection system breadboard to establish its operational status. This section contains specifically defined system and modular limitations, recommended improvements and changes, and defined and recommended design approach that permits a more rapid responding system.

HARDWARE OPERATIONAL STATUS

The breadboard system is disassembled into its modular components.

The detector module has been disassembled permanently for component salvage and use on other projects. Samples of the TNT_{ase} reaction cell and the luminescence reaction cell are available for examination, inspection, and disassembly. Complete level 1 documentation of the breadboard reaction cell hardware in the breadboard is included. Procedures for maintenance, purification, assay, and immobilization of the TNT enzyme are described in Appendix I of this report. Procedures for the growth and purification, assay, immobilization, and co-immobilization of the oxidoreductase and the bacterial luciferase enzyme in the luminescence reaction cell are also described in Appendix I of this report. All equipment, chemicals, and detector components are described in Appendices E, F, G, and H. The TNT-nitroreductase deposit is documented in Appendix D.

Information for the complete replication of the TNT vapor detection system breadboard or any of its modules is included in this report.

PERFORMANCE ASSESSMENT

All aspects of the breadboard and module performance have been assessed experimentally or have been evaluated by engineering analysis. Table 2 (page20) is a compilation of the system and modular performance parameters. Long-term drift, span and zero, linearity, ambient temperature variation drifts, and interferent equivalent response information are lacking. However, sufficient information is available to obtain preliminary estimates of false-alarm and missed-alarm probabilities from system instabilities at a preselected alarm set point concentration level.

The author believes that the sensitivity performance limits listed in Table 2 are established by the design limitations of the detector module. Time rate-of-response characteristics are limited by the overall modular design approach of the sample train and detector. To significantly reduce the system time rate-of-response, an integrated sample train-detector module concept is required as described in Section VI.

REDUCTION OF SIGNAL ENHANCEMENT AND NOISE

Several independent design changes can be made to the breadboard system to enhance the signal and improve the MDC. The author performed a cursory examination of the system zero signal under different conditions and believes that at least an order of magnitude reduction in noise can be achieved by the changes described in the following sections. Other more fundamental changes can be made to augment the signal further, as described in the following paragraphs.

Pump Replacement

Because the signal is proportional to the reduction in light level intensity, the most significant noise factor ($f \simeq 1$ Hz) arises from variation in the light output of the luminescence reaction cell. It was determined that the main source of noise at this stage of the development was the unsteady flow of reagent and sample liquid streams supplied by a peristaltic pump. Certainly a more steady stream flow can be obtained from gear or syringe pumps. The author believes the noise arising from the peristaltic pump can be eliminated entirely so that the main source of noise remaining will be variations in the luminescence intensity arising from the nonuniform and unsteady local liquid stream flow in the luminescence reaction cell packed bed.

Relocation of Luminescence Reaction Cell

An important change is the relocation of the reaction cell closer to the light detector face. In the the Center's breadboard configuration, it is approximately 8.8 mm from the reaction cell window. The reaction cell window is 2.2-mm thick, and there is a 2-mm gap between the window and the light detector face. Thus, the total separation between the reaction cell and the light detector face is 13 mm. Moving the reaction cell in its current configuration to within 2 mm of the light detector face could possibly increase the signal by a factor of 42.3. It is very probable, however, that the noise level will increase by a comparable factor unless changes are made simultaneously to reduce the noise sources, e.g., the pump.

Redesign of the Luminescence Reaction Cell

Sufficient reduction of the liquid pump noise will shift the largest source of noise to localized liquid flow rate variations and the resulting light output variations from the bed of the reaction cell. A redesign of the luminescence reaction cell will be required to reduce these variations. A reaction cell with a large flow cross-sectional aspect ratio and a more uniformly packed cell will produce liquid flow with a more uniform velocity across the bed to minimize localized reaction noise.

In addition, this cell design will also provide a larger signal because a reaction with a large cross-section aspect ratio exposes more of the packing reaction sites to the face of the light detector.

Examination of Light Detector

The changes described in the previous paragraphs may reduce the noise level arising from the luminescence reaction cell to levels comparable to that of noise arising from the light detector itself. In that event, a change in the light detector (photomultiplier tube) from that used by the breadboard system to a more sensitive and/or less noisy photomultiplier tube may be fruitful. In addition, if the reaction cell and photomultiplier tube noise are made approximately equal, then the use of a more sensitive or less noisy photomultiplier tube or a cooled photomultiplier tube will subsequently reduce the overall noise by 0.7. For example, if the photomultiplier tube noise is two times the reaction cell noise and if it is reduced by the same technique, the reduction in overall noise is approximately 0.44. Thus, the overall signal-to-noise increase by the changes described earlier can approach two orders of magnitude after elimination of the pump noise source.

The author believes that implementation of the design changes listed above will result in a detection system with a 0.0025-ppt (v/v) TNT-in-air MDC over a 10-minute total analysis time. Further large reductions in the system MDC are probably only achievable by changing the enzyme chemistry such that the luminescent reaction, reaction (9) of Section IV, is independent of oxygen, and the luminescence product is proportional directly to the TNT or explosives molecule concentration. In other words, the signal is not proportional to the reduction to the high background level as it is in the current breadboard system but proportional directly to the luminescence intensity produced by the TNT molecules in the sample stream.

SYSTEM DESIGN CONCEPT FOR RAPID TIME RATE-OF-RESPONSE

The breadboard system time rate-of-response comprises two components: the sampling period (> 10 minutes) and the detector analysis time (approximately 103 seconds). It is evident that MDC is almost a direct trade-off with sampling time. Thus, development of a more sensitive detector can reduce the sampling time directly. For example, a tenfold increase in the detector module signal-to-noise ratio can be traded to reduce the sampling time to < 2 minutes while maintaining the current system MDC. The author believes that large reductions in the system time rate-of-response to less than 1 minutes and MDC better than 0.001 ppt (v/v) can only be achieved by a radical design that integrates the sample train and detector into a single module operating on a continuous basis and not incrementally as does the breadboard.

A preliminary conceptual basis for such a continuous detection system is depicted by Figure 10. It illustrates the concept only and provides some insight as to type of equipment and fundamental engineering developments required to obtain the system. In this design, the

reaction cells or reaction beds are transported to the sample and reagent streams. Otherwise, it is identical to the breadboard system of Figures 3 through 6.

The reaction cell comprises a thin ribbon of reasonably porous material on which the TNTase, oxyreductase, and bacterial luciferase enzymes are co-immobilized. A roll of moist reaction ribbon (approximately 6 inches wide) is stored in the collection ribbon supply container. It moves and is threaded through the sampling scoop, reagent rollers, light detector compartment, and finally to the spent ribbon container and drive. During operation, the reaction ribbon is pulled through the sample train. Sample air flows through the scoop at a high volumetric flow rate (20,000 L/minute). The scoop has a bell-mouth 12-inch diameter inlet and a 6-inch diameter throat. With the reaction ribbon moving at a 1-inch/second linear velocity, it is exposed to the sample air for 6 seconds and collects sample by absorption from approximately 2000 L of air.

A vacuum blower at the sampling scoop outlet moves air at 20,000 L/minute through the reaction ribbon. As the reaction ribbon emerges from the sampling scoop, it passes through a thin gap between two reagent injection rollers, which load the reaction ribbon with the reagent fluids. A light-tight compartment containing the enzyluminescence detector receives the reaction ribbon from the reagent injection rollers to provide an alarm.

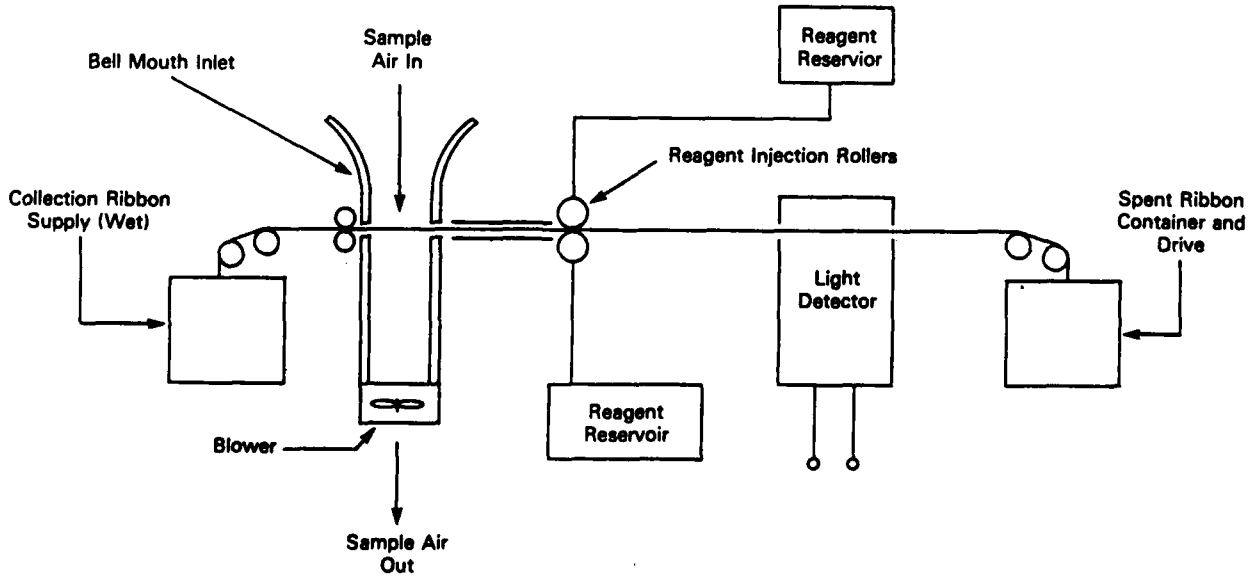

Figure 10. Conceptual Continuous Detection System

SECTION VII
VAPOR DETECTION SYSTEM APPLICATIONS ANALYSIS

Although the enzyluminescence TNT vapor detection system has been suspended as an antitank mine detection device, the author believes that the technology and engineering developed by the program is of significant utility for other applications. An examination of the user requirements and performance specifications of Table 1 (page10) shows that with the performance specifications of the current BRDEC breadboard detection system and an MDC improvement by a factor of 250, the system can be applied adequately as a room or building air TNT vapor monitor. It was shown in Section VI, Engineering Analysis, that an MDC improvement of at least two orders of magnitude can be obtained by component and design changes to the current system.

TNT VAPOR PROPAGATION IN A ROOM OR BUILDING

It is evident that the most important detection system requirement in room and/or building monitoring applications is the system MDC. An analysis of a simple TNT mass transport model is used to estimate the TNT vapor concentration in a room or building under typical circumstances and to aid in defining detection operational procedures. A ventilated room containing a TNT mass emitting TNT vapors at a constant rate is examined to determined the room air average TNT concentration.

The TNT vapors accumulate in the room air. Most, however, are adsorbed to surfaces. Those that remain in the mass of room air are carried out with the ventilation air stream. This relationship is expressed in terms of a TNT vapor material balance:

$$V_r\,(dC/dt) = q_{TNT} - Q_x - Q_s \qquad (10)$$

where

- C = room air average TNT vapor mole fraction concentration, dimensionless;
- t = time, second;
- V_r = room air space volume, cm^3;
- q_{TNT} = TNT mass TNT vapor emission rate, cm^3/second;
- Q_x = TNT vapor removed from the room air with the ventilating air, cm^3/second; and
- Q_s = TNT vapor adsorption rate to surfaces, cm^3/second.

Furthermore,

$$Q_x = Q_{ex}C \text{ and } Q_s = k_m A_s C \tag{11}$$

where

Q_{ex} = room air exchange rate, cm^3/second;
k_m = TNT molecule average mass transfer coefficient to surfaces, cm^3; and
A_s = room total adsorption surface area, cm^2.

A combination of these equations yields:

$$V_r\,(dC/dt) = q_{TNT} - Q_{ex}C - k_m A_s C \tag{12}$$

or

$$C = [q_{TNT}/(Q_{ex} + k_m A_s)]\,(1 - e^{-\alpha t}) \tag{13}$$

$$\alpha = (Q_{ex} + k_m A_s)/V_r$$

In this relationship, α is the reciprocal time constant, s^{-1}.

Equations (10) and (11) are valid for the conditions defined by the following assumptions:

(1) TNT vapor molecules are released into the room air at a constant rate;

(2) TNT vapor molecules are distributed uniformly in the room air;

(3) The adsorption surfaces contained within the room are identical in that the TNT vapor molecule sticking coefficient is unity;

(4) The TNT vapor molecule mass transfer coefficients from the air to each adsorption surface are identical; and

(5) The adsorption surfaces are never saturated with adsorbed TNT molecules.

Under equilibrium circumstances at constant temperature, Assumptions 1 and 3 are reasonable[16, 18] (Pate, 1976; Peterson and Conrad, 1978).

Assumption 2 implies that sufficient air circulation, forced or free, exists to help in rapidly and uniformly distributing the TNT vapor molecules from the point of origin into the room air. Over short time periods, it may be somewhat fallacious to assume a uniform TNT vapor concentration in a room. However, to avoid mathematically accounting for TNT vapor concentrations in the room air and to obtain a first-order approximation of TNT vapor concentration in the room, Assumption 2 is taken as valid.

Assumption 4 is valid only in regions of the room where the aerodynamic conditions over adsorbing surfaces are identical. k_m varies proportionally with air velocity or more precisely with the air-surface Reynolds number[12] (Lucero and Boncyk, 1986). However, Assumption 4 is taken as valid to avoid mathematically accounting for variations in the circulating air velocity in the room and again to obtain a first-order approximation of TNT vapor concentration in the room containing adsorbing surfaces.

Assumption 5 is equivalent to stating that surface adsorption saturation is obtained only after many layers of TNT molecules are adsorbed. The mass adsorption capacity of the surface is always in excess of the amount of TNT vapors released by the TNT source into the room at any given time.

The author has chosen to deal with the case described by equations (10) and (11) and the attendant assumptions to examine the transport and accumulation of TNT vapor molecules in the air of a room or building. This examination aids in illustrating the basic detection problems, suggests detection procedures, and promotes perceptive questions regarding detection system hardware and its utility.

Room Conditions and Transport Parameters

The parameters comprising the coefficients of the terms in equations (10) and (11) establish and define the room conditions and TNT vapor transport parameters. The author arbitrarily defined the room and room conditions and used standard engineering practices and data available for conditions similar to those described by the assumptions previously listed. Table 3 lists the room conditions and transport used in the evaluation of equation (13).

Table 3. Room Conditions and TNT Transport Parameters

Room Conditions

Dimensions: 9.14 x 9.14 x 3.05 m (30 x 30 x 10 ft)
Air Exchange Rate: 1/hr
Total Exposed Surface Area: 1000 times the floor, ceiling, and wall projected surface area

$V_r = 2.55 \times 10^8$ cm^3

$Q_{ex} = 7.1 \times 10^4$ cm^3/s

$A_s = 2.8 \times 10^9$ cm^2

TNT Source Emission Rate (Pate, 1976)

$q_{TNT} = 1.7 \times 10^{-8}$ cm^3/s

Mass Transport Parameters

The TNT vapor convective mass transfer coefficient (k_m) is the ratio of the TNT diffusion coefficient (D, cm^2/s) in air to the air boundary thickness attendant to the adsorbing surface (δ, cm). For laminar flow over a surface with a 1-s air dwell time and a 0.043-cm^2/s TNT in air diffusion coefficient (Griffy, 1989),

$\delta = 0.2$ to 0.5 cm

Therefore,

$k_m = 0.22$ to 0.09 cm/s

Room TNT Vapor Concentration at Equilibrium

Equilibrium conditions are attained at t = 0 in equation (13). Thus,

$$C = q_{TNT}/(Q_{ex} + k_m A_s) \qquad (14)$$

and for

$q_{TNT} = 1.7 \times 10^{-8}$ cm^3/s
$Q_{ex} = 7.1 \times 10^4$ cm^3/s
$k_m = 0.22$ to 0.09 cm/s
$A_s = 2.8 \times 10^9$ cm^2
$k_m A_s = 6.16 \times 10^8$ to 2.52×10^8

then

$C = 2.76 \times 10^{-5}$ to 6.75×10^{-5} ppt

This is a very low concentration. Note the dominance of the $k_m A_s$ term over Q_{ex}, i.e., $k_m A_s / Q_{ex} = 10^4$. Only $k_m A_s$ can be manipulated to increase C. It is accomplished by creating or establishing conditions by which k_m and/or A_s are made smaller. k_m can be made much smaller by a 0.01 factor by reducing the air circulation in the room. This suggests that following procedural and detection hardware constraints:

- Maintain $Q_{ex} = 0$ by shutting down fans and air conditioning,
- Minimize disturbances to the room air by minimizing the number of people used in the detection process,
- Minimize the sample air intake and exhaust velocity, or
- Remove room articles (furniture, etc.) to minimize A_s.

Thus, if k_m can be reduced by a 0.01 factor, then

$$C \cong 3 \times 10^{-3} \text{ ppt}$$

Time to Attain Room TNT Vapor Concentration Equilibrium

The time constant for equation (13) is

$$t_c = V_r / (Q_{ex} + k_m A_s)$$

and the time to attain 99 percent equilibrium is

$$T_{eq} = 5\, t_c = 5\, V_r / (Q_{ex} + k_m A_s)$$

For the conditions of Table 3,

$$T_{eq} = 2.48 \text{ to } 6.1 \text{ s}$$

This is a very short time period owing entirely to the large $k_m A_s$ product. Reverting to the appears unreasonable, nonetheless, in view of some experimental results obtained for the TNT procedures and suggestions listed earlier, T_{eq} is increased to 248 seconds or 4.13 minutes. This time emissions from luggage pieces[16] (Pate, 1976). The author believes that this discrepancy arises primarily from the assumption in equation (13) that at $t = 0$, q_{TNT} is at equilibrium. Thus, the time to attain equilibrium may be viewed as two segments: a long time period to establish a steady-state q_{TNT} and a much shorter time period to establish C_{eq} in the room air.

TNT DETECTION

The estimates of room TNT vapor concentration and equilibrium suggest the practical aspects of TNT vapor detection in a room. These are detection scenarios by a stationary device and one by a portable or transportable device within the room.

Vapor Detection by a Stationary System

It is probable that the MDC requirements for a stationary detection system will be more stringent because more room air circulation is required to perform the detection, which increases the product $k_m A_s$ and correspondingly reduces C_{eq} (approximately 10^{-3} to 10^{-5} ppt) of equation (13). For the Center's system, and many others for that matter, long preconcentration periods will be required. For example, a corresponding preconcentration period of approximately 10 minutes to 2 hours is required to perform a single detection analysis cycle. To obtain a more practical or shorter cycle time, the air handling capacity of the Center's system preconcentrator must be increased by 1 or 2 orders of magnitude, or a long heated (approximately 160°C) sampling hose should be used to permit the hose inlet to be used as a portable sampling probe. Without transport TNT vapor losses, this operating condition is nearly equivalent to using a portable or transportable system.

Vapor Detection by a Portable or Transportable System

It is certain that a portable or transportable detection system with a 0.001 ppt MDC can perform a detection with a lower missed-alarm probability and with more convenience to the user.

DETECTION PROCEDURES

The author believes that successful room air TNT vapor detection by means of a detection system with limited sensitivity (MDC $\cong$ 0.001 to 0.0001 ppt) can be augmented with proper detection operational procedures. The applications analysis of this section is only a cursory treatment that yields valuable information in this regard. Despite the bold assumption taken in the derivation of equation (10) and its parametric evaluation, the author believes that these are much more prudent than reckless. Based on the information learned by this application analysis and other analyses that may be performed by users, the author advises the detection system user to

- Develop detection plans and procedures for defined circumstances and conditions;
- Assess the circumstances and conditions of the detection scene prior to the detection episode; and
- Match the detection scene circumstances and conditions to the closest defined circumstances and conditions and implement the corresponding plans and procedures.

REFERENCES

1. *Reassessment Survey Report on Explosives Detection and Identification Techniques*, Report ATR 77(7911-04)-1, Aerospace Corporation, 1977.

2. C. Bryant and T. Martin, University of California, San Diego, Bimonthly Progress Report, Contract No. DAAK-70-86-K-0031, June 1987.

3. M. DeLuca, and D. Vellom, 1982, *Detection of Low Levels of TNT Using Immunologic and Bioluminescent Techniques*, Report No. DAAK 70-79-C-0174, University of California at San Diego for U.S. Army Mobility Equipment Research and Development Command, Fort Belvoir, Virginia.

4. H. C. Egghart, 1978, in Proceedings, *New Concepts Symposium and Workshop on Detection and Identification of Explosives*, NTIS No. PB290055, Washington, D.C., p. 169.

5. H. C. Egghart, *The MERADCOM In Vitro Biosensor Program*, Report 2364, U.S. Army Mobility Equipment Research and Development Command, Fort Belvoir, Virginia, June 1982.

6. T. A. Griffy, *A Model of Explosives Vapor Concentration*, Proceedings, Third Symposium on Analysis and Detection of Explosives, Mannheim, Germany, p. 38-1, 1989.

7. D. P. Lucero, *Symposium on Explosives Detection for Security Applications*, J. Testing and Evaluation 13, p. 210, 1985.

8. L. J. Kricka and D. Vellom, Methods in Enzymology 133, p. 229, 1986,.

9. L. J. Kricka, G. K. Wienhauser, J. E. Hinkley, and M. DeLuca, Analytical Biochemistry 129, p. 392, 1983.

10. D. P. Lucero, J. Chromat. Sci. 23, p. 293, 1983.

11. D. P. Lucero, *User Requirement and Performance Specifications for Explosives Vapor Detection Systems*, J. Testing and Evaluation 13, p. 222, 1985.

12. D. P. Lucero and E.M. Boncyk, *Transport and Preconcentration of Explosives Vapors by Liquid Sampling Modules*, J. Energetic Mat 4, p. 473, 1986.

13. J. McBride, Philadelphia Police Department, Philadelphia, Pennsylvania, private communication with E.M. Boncyk, U.S. Army Belvoir RD&E Center, Fort Belvoir, Virginia, April 1989.

14. MRI, 1985, *Design and Develop a Vapor/Liquid Interface*, Midwest Research Institute for U.S. Army Belvoir R&D Center Procurement and Production Directorate, Fort Belvoir, Virginia, Contract No. DAAK70-83-C-0068, August 3, 1985, to August 3, 1986.

15. MRI, *Design and Develop a Vapor/Liquid Interface*, Midwest Research Institute Final Report, 1988.

16. C. T. Pate, *Characteristics of Vapors Emanating from Explosives*, LEAA Contract J-LEAA-025-73, Analytical Research Laboratories, Inc, 1976.

17. P. A. Pella, Anal. Chem. 48, p. 1632, 1976.

18. P. K. Peterson and F.J. Conrad, in Proceedings, New Concepts Symposium and Workshop on Detection and Identification of Explosives, p. 85, 1978.

19. Proceedings, New Concepts Symposium and Workshop on Detection and Identification of Explosives, 1978, NTIS No. PB290055, p. 37.

20. A. H. P. Skelland, *Diffusional Mass Transfer*, John Wiley and Sons, pp. 266-268, 1974.

21. L. Stryer, *Biochemistry*, 3rd edition, W.H. Freeman and Company, New York, pp. 187-195, 1988.

22. D. Vellom, J. Hinkley, A. Loucks, H. Egghart, and M. DeLuca, *Continuous Flow Bioluminescent Assays for Femtomole Levels of NADH and TNT*, Analytical Applications of Bioluminescence and Chemiluminescence, The Academic Press, Inc., p. 133, 1984.

23. G. K. Wienhauser, L. J. Kricka, J. E. Hinkley, and M. DeLuca, Applied Biochemistry and Biotechnology 7, p. 463, 1982.

24. W. D. Williams and J.K. Syverson, *Explosives Detector Field Tests*, Research and Development Division, Bureau of Alcohol, Tobacco and Firearms, U.S. Department of the Treasury, Washington, D.C., 1981.

APPENDIX A
BELVOIR RESEARCH, DEVELOPMENT AND ENGINEERING CENTER'S DEVELOPMENT CONTRACT CHRONOLOGY

Project Title	Contract Number	Period Covered	Contractor
Bioluminescence Explosive Detector	DAAK02-72-C-0615	June 1971 - January 1974	RPC Corporation Segundo Beach,CA
Mechanism of Bioluminescent Detection of Explosive Vapors	DAAK02-72-C-0614	June 1972 - April 1974	Midwest Research Institute Kansas City, MO
Detection of Explosive Vapor by Enzymatic Methods	DAAK02-74-C-0100	December 1973 - December 1977	Beckman Instruments, Inc. Carlsbad, CA
Characterization of the Component Enzymes of the TNT Detection System—Physical Chemical Studies	DAAK70-77-C-0129	August 1977 - June 1979	Beckman Instruments, Inc. Carlsbad, CA
Detection of Low Levels of TNT Using Immunologic and Bioluminescent Techniques	DAAK70-79-C-0174	October 1979 - September 1982	University of California San Diego LaJolla, CA
Detection of Low Levels of TNT Using Immunologic and Bioluminescent Techniques	DAAK70-83-K-0003	October 1982 - September 1985	University of California San Diego LaJolla, CA
Design and Develop a Vapor/Liquid Interface	DAAK70-83-C-0068	June 1983 - May 1988	Midwest Research Institute Kansas City, MO
Detection of Low Levels of TNT Using Immunologic and Bioluminescent Techniques	DAAK70-86-K-0031	April 1986 - March 1989	University of California San Diego LaJolla, CA

APPENDIX B
CONTRACT DOCUMENTATION

Contract Number	Contractor	Monthly Reports	Interim Reports	Final Report	Location of Reports
DAAK02-72-C-0615	RPC Corporation	Unknown	Unknown	Yes	Defense Technical Information Center Alexandria, VA
DAAK02-72-C-0614	Midwest Research Institute	Unknown	Unknown	Yes	Defense Technical Information Center Alexandria, VA
DAAK02-74-C-0100	Beckman Instruments, Inc.	Unknown	Yes	Yes	Belvoir Research, Development, and Engineering Center Ft. Belvoir, VA
DAAK70-77-C-0129	Beckman Instruments, Inc.	Unknown	Unknown	Yes	Belvoir Research, Development, and Engineering Center Ft. Belvoir, VA
DAAK70-79-C-0174	University of California San Diego	Yes	No	Yes	Belvoir Research, Development, and Engineering Center Ft. Belvoir, VA and Univ. of Calif. SD LaJolla, CA
DAAK70-83-K-0003	University of California San Diego	Yes	Yes	Yes	Belvoir Research, Development, and Engineering Center Ft. Belvoir, VA and Univ. of Calif. SD LaJolla, CA
DAAK70-83-C-0068	Midwest Research Institute	Yes	Yes	Yes	Belvoir Research, Development, and Engineering Center Ft. Belvoir, VA and Midwest Research Inst. Kansas City, MO
DAAK70-86-K-0031	University of California San Diego	Yes	No	In Progress	Belvoir Research, Development, and Engineering Center Ft. Belvoir, VA and Univ. of Calif. SD LaJolla, CA

APPENDIX C
CONTRACT OPEN PUBLICATIONS

1. H.C. Egghart, *The MERADCOM In Vitro Biosensor Program*, Report 2364, U.S. Army Mobility Equipment Research and Development Command, Fort Belvoir, Virginia, June 1982.

2. D.P. Lucero and E.M. Boncyk, *Transport and Preconcentration of Explosives Vapors by Liquid Sampling Modules*, J. Energetic Mat 4, 1986, p. 473.

3. D. Vellom, J. Hinkley, A. Loucks, H. Egghart, and M. DeLuca, *Continuous Flow Bioluminescent Assays for Femtomole Levels of NADH and TNT*, Analytical Applications of Bioluminescence and Chemiluminescence. The Academic Press, Inc., 1984, p. 133.

4. L.J. Kricka and D. Vellom, *Methods in Enzymology 133*, 1986, p. 229.

5. H.C. Egghart in Proceedings, New Concepts Symposium and Workshop on Detection and Identification of Explosives, NTIS No. PB290055, Washington, D.C., 1978, p. 169.

6. L.J. Kricka, G.K. Wienhauser, J.E. Hinkley, and M. DeLuca, Analytical Biochemistry 129, 1983, p. 392.

7. G.K. Wienhauser, L.J. Kricka, J.E. Hinkley, and M. DeLuca, Applied Biochemistry and Biotechnology 7, 1982, p. 463.

8. E.M. Boncyk and D.P. Lucero, "Bioluminescent TNT Detection System," in Proceedings of International Congress on Applied Criminology, Gent, Belgium, 1988.

9. R.V. Nolan, H.C. Egghart, L. Mittleman, R.L. Brooke, F.L. Roder, and D.L. Gravitte, *MERADCOM Mine Detection Program: 1960-1980*, Report 2294, U.S. Army Mobility Equipment Research and Development Command, Fort Belvoir, Virginia, 1980.

10. E.M. Boncyk, "A Bioluminescent Explosives Vapor Detection and Identification System," in Proceedings of the 3rd International Symposium on the Analysis and Detection of Explosives, Mannheim, Germany, 1989, p. 40-1.

11. J. Wannlund, J. Azari, L. Levine, and M. DeLuca, *A Bioluminescent Immunoassay for Methotrexate at the Subpicomole Level*, Biochemical and Biophysical Research Communications, 96, 1, Academic Press, September 1980, p. 440.

12. J. Wannlund and M. DeLuca, *Bioluminescent Immunoassays,* Methods in Enzymology 92, Academic Press, 1983, p. 426.

13. J. Wannlund and M. DeLuca, *Bioluminescent Immunoassays: Use of Luciferase-Antigen Conjugates for Determination of Methotrexate and DNP*, Bioluminescence and Chemiluminescence, Academic Press, 1981, p. 693.

14. J. Wannlund and M. DeLuca, *A Sensitive Bioluminescent Immunoassay for Dinitrophenol and Trinitrotoluene*, Analytical Biochemistry 122, Academic Press, 1982, p. 385.

15 J. Wannlund, *Firefly Luciferase: Studies of the Mechanism and the Development of a Bioluminescent Immunoassay*, Doctor of Philosophy in Chemistry Thesis, University of California San Diego, 1982.

16. J. Grimes, R. Kemp, and R. Sakaida, *Bioluminescence Explosive Detector*, RPC Corporation, Contract DAAK02-72-C-0615, DTIC AD528634, January 1974.

17. L.H. Goodson, W.B. Jacobs, and F.E. Wells, *Mechanism of Bioluminescent Detection of Explosives Vapors*, Midwest Research Institute, Contract DAAK02-72-C-0614, DTIC AD920913L, June 1974.

APPENDIX D
DOCUMENTATION OF THE TNT-NITROREDUCTASE DEPOSIT AT THE AMERICAN TYPE CULTURE COLLECTION, ROCKVILLE, MARYLAND

American
Type Culture
Collection
12301 Parklawn Drive • Rockville, Maryland 20852 USA
Telephone: (301) 881-2600 • Telex ATCCROVE 908-768

Do not write in this box
ATCC Number
Accession Date
Date Received

COLLECTION OF BACTERIA

To be completed by depositor of strain. Please print or type.

1. Scientific name of organism PSEUDOMONAS~
2. Strain designations other than ATCC number STRAIN 96-3
3. Is this the type strain of this organism (see reverse side)? _____ If this strain has been designated in the literature as the type strain, please cite reference: _____
4. Isolated by BECKMAN INSTRUMENTS, INC. MECHONICS OPERATIONS
   from NATURALLY TNT(TRINITROTOLUENE) ENRICHED SOIL date 1974
5. If you did not isolate this strain indicate from whom you received it:
   ATCC – depositor – BECKMAN INSTRUMENT, INC. –
6. Reason for deposit: requested by ATCC _____, new taxon: species _____, subspecies _____
   produces the antibiotic _____ assay of α - TRINITROTOLUENE
   production of NITRIC REDUCTASE (NITRIC-AZYL REDUCTASE) other _____
7. Maintenance:
   Medium (attach formula) _____
   Temperature 30°C Other _____
8. Does this organism survive: Freeze-drying? UNDETERMINED Freezing? -70°C (YES)
   Recommended method for long-term preservation: -70°C IN 7% DMSO SOLUTION OF GROWTH MEDIUM
9. Is this strain zoopathogenic? NO If so, would you classify it as class 2, 3, or 4? _____
   (see reverse side for description of classes)
10. Is this strain phytopathogenic? NO (Information required by Plant Quarantine Division, USDA) If so,
    a. Is the geographical distribution of this organism general, limited, or unknown (encircle)?
    b. Would you recommend that this strain be made available to any qualified investigator regardless of his location? YES
    c. If not, what limits would you place on the distribution of this strain?
11. Please attach a complete description of this strain unless description is given in accompanying reprint.
12. References (Please enclose two of each, if available):
    _____
    _____
13. Comments: _____
    _____
    _____
14. *I understand that this material is for deposit in the ATCC general collection. It will be examined, and if accepted by the ATCC, batches will be made and distributed to the scientific community for a fee to cover expenses.*
    12/1/96 (Date) M. De Luca (Signature of authorized individual)
    Deposited on Behalf of DR. MARLENE DELUCA
15. Depositor Address UNIV. OF CALIF., SAN DIEGO; DPT. OF CHEMISTRY, M-001,
    LA JOLLA, CA. 92093

TYPE STRAINS

For each named taxon, a nomenclatural type must be designated. The nomenclatural type of a species (and of a subspecies) is a strain, which may be represented by one or by any combination of the following: a description, illustration, or preparation, but preferably by living cultures. The type strain of a named species or subspecies must be the strain (or one of the strains) on which the original description of the organism was based. The author who first publishes a name for an organism should designate, in the original publication, the type strain. If he fails to do this, any subsequent author may designate in the literature one of the original strains as the type. If none of the strains on which the original description is based are extant, a neotype strain may be designated.

A neotype strain is a strain accepted by international agreement to replace a type strain which is no longer in existence. The neotype should possess the characteristics as given in the original description; any deviations should be explained. A neotype strain must be proposed by an author in the *International Journal of Systematic Bacteriology* (IJSB) together with a reference (or references) to the first description of the proposed neotype strain, and a record of the author's designation for the neotype strain and of at least one culture collection from which cultures of the neotype strain are available. The neotype becomes established from the date of publication in the IJSB. Any objections should be referred to the Judicial Commission. A neotype strain shall be proposed only after a careful search for original strains. If an original strain is subsequently discovered, the matter shall be referred immediately to the Judicial Commission.

If neither a type nor a neotype strain exists for a named species or subspecies, the original description of the organism may serve as the nomenclatural type.

ATCC Classification of Agents

Because of the great variety of microorganisms distributed by the American Type Culture Collection, the ATCC and its advisory committees, the Public Health Service, United States Department of Agriculture, and other cooperating agencies have classified organisms according to pathogenicity into four groups for purposes of packaging for safe shipment.

Class 1 consists of agents of no recognized hazard under ordinary conditions of handling. Unrestricted distribution can be made to any *bona fide* teaching, research or industrial institution.

Class 2 consists of agents of ordinary potential hazard. Distribution is restricted to professional investigators in departments of microbiology in universities, in hospitals, in research and industrial institutions, and also to such personnel in diagnostic laboratories dealing with problems of infectious disease. Examples of these agents are *Staphylococcus, Candida albicans* and measles virus. Requests for these agents should be made on the institution's official purchase order or official stationery.

Class 3 consists of pathogens involving special hazard. Distribution is restricted to qualified professional investigators. Requests for these agents should be made on the institution's official stationery or purchase order and should be signed by the director of the institution, the chairman of the department concerned, or an official of equivalent rank. Examples of these agents are *Brucella, Histoplasma,* and Western Equine Encephalitis virus.

Class 4 consists of agents of potential danger to the public health and/or animal health or of extreme hazard to laboratory personnel requiring special facilities for their containment. Examples of these agents are *Pseudomonas pseudomallei* and Venezuelan Equine Encephalitis virus. Distribution can be made only after the required permit is obtained.

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Univ. of California,San Diego
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La Jolla, CA 92093

American Type Culture Collection
12301 Parklawn Dr.
Rockville, Maryland 20852

Adendum to ATCC Deposit Sheet for Bacteria

A. Growth Conditions of Strain 96-3:

The composition of the mediun used at this time is as follows: (per 1000 ml H_2O)

25 mM potassium phosphate buffer, pH 7.0 (25 ml of 1 M stock)
2 g NH_4NO_3
0.2 g $MgSO_4*7H_2O$
10 g Difco yeast extract
1% glucose (20 ml of a 50% stock previously autoclaved)
1 ml of trace salts solution -
0.12% $FeSO_4*7H_2O$
0.12% $ZnSO_4*7H_2O$
0.104% $MnCl_2*4H_2O$
0.5% NaCl

To prepare 4 liters of strain 96-3 for subsequent harvest, we begin by plating a $1:10^{-5}$ dilution of a frozen (-70°C) Stock.Plates are of the above medium with 1.5% agar added. A single colony is picked and a 10ml culture is grown overnight. From this culture four 25 ml 1% starter cultures were prepared and grown overnight in the above medium at 30°C with constant shaking. These starter cultures were added directly to four one liter flasks of culture medium. If induction of the TNT nitro-aryl reductase is desired 50mg per liter per hour is added to the growth medium.

B. Description of Microorganism 96-3:

Microorganism 96-3 was initially isolated by Beckman Instruments, Inc. Microbics operation in 1974. The microorganism is a Gram Negative, rod shaped bactria which exhibits motility. The only resistance thus far encountered is to penecillin. 96-3 shows abnormally good growth characteristics in the presence of 50 to 100mg of 2,4,6-Trinitrotoluene and under these conditions expresses an enzyme which metabolizes this compound to some extent.

SINCE 1925 AN INDEPENDENT NON-PROFIT ORGANIZATION DEVOTED TO
THE PRESERVATION AND DISTRIBUTION OF REFERENCE CULTURES

American
Type Culture
Collection

12301 PARKLAWN DRIVE · ROCKVILLE, MARYLAND 20852-1776 USA · (301) 881-2600 · TELEX ATCCROVE 908-768

September 19, 1986

Dr. Chris Bryant
Department of Chemistry, M-001
University of California San Diego
La Jolla, CA 92093

Dear Dr. Bryant:

Thank you for your telephone call of September 19, 1986. We should be very pleased to consider your culture for addition to the American Type Culture Collection. We would also like to have any reprints or preprints pertaining to this organism.

We would prefer receiving a lyophilized culture but if unavailable, the organism may be sent as a test tube culture in the appropriate medium and adequately labeled. We would also appreciate receiving, in advance, any specific cultural requirements for your strain so that we may prepare for the receipt and subsequent processing of your culture.

After processing is completed, we will send you two of our freeze-dried preparations: one for your approval and one for your stocks.

Enclosed is an ATCC Form 1-B to be completed in connection with your deposit. Upon receipt of the culture and the completed form, we will notify you concerning the deposition of your culture.

Sincerely yours,

Ellen L. Baque

(Mrs.) Ellen L. Baque, Secretary
Department of Bacteriology

enb

Enclosure

Date 1-9-87

Dear Depositor:

The following culture(s) arrived and have been assigned the following ATCC accession number(s):

Pseudomonas sp. 96-3 ATCC 43560

After processing is completed, we will send you two of our freeze-dried preparations: One for your approval and one for your stocks.

Thank you. Signed Ellen L. Sague Secretary

Bacteriology Department
American Type Culture Collection
12301 Parklawn Drive
Rockville, Maryland 20852

APPENDIX E
CHEMICALS DESCRIPTION

Item	Stock Number	Vendor	Quantity	Price
Trinitrotoluene (TNT) (70 - 95%)	8515C	Chem Service, Inc. 215-692-3026 P.O. Box 3108 West Chester, PA 19381	1gm	8.83
β-Nicotinamide-adenine dinucleotide, oxidized form (NAD)	127973	Boehringer Mannheim Biochemicals 800-262-1640 P. O. Box 50816 Indianapolis, IN 46250	5gm	100.00
β-Nicotinamide-adenine dinucleotide, reduced form (NADH)	837075	BMB	2gm	120.00
β-Nicotinamide-adenine dinucleotide, oxidized form (NADP)	236659	BMB	100gm	35.00
β-Nicotinamide-adenine dinucleotide, reduced form (NADPH)	107824	BMB	100gm	54.00
Dithiothriotol (DTT)	100032	BMB	5gm	45.00
Flavin mononucleotide (FMN)	476501	BMB	1gm	22.00
4-Morpholinethansulfonsaure (MES)	223794	BMB	100gm	32.00
Tris(hydroxymethyl)aminomethane (Tris)	604205	BMB	1gm	40.00
Acetone ChromAR. HPLC grade	2435-050	Mallinckrodt 800-354-2050 Science Products Division Paris, KN 40361	1 liter	16.35
Acetonitrile ChromAR.HPLC grade	2856-050	Mallinckrodt	1 liter	27.75
Methyl Alcohol ChromAR. HPLC grade	3041-050	Mallinckrodt	1 liter	14.50
Bovine Syrum Albumin. Fraction V Powder (BSA)	A 2153	Sigma Chemical Company 800-325-3010 P. O. Box 14508 St. Lewis, MO 263178	5g	11.70
Ethylenediaminetetraacetic acid. Free acid (EDTA)	EDS	Sigma	100g	7.05
2-Mercaptoethanol	M 6250	Sigma	25mL	4.40
Potassium Carbonate (K_2CO_3)	P4379	Sigma	100g	4.80
Potassium Chloride (KCl)	P4504	Sigma	250g	5.65
Potassium Hydroxide (KOH)	P1767	Sigma	250g	5.75
Potassium Phosphate Monobasic	P5379	Sigma	100g	7.15
Sodium Azide (NaN_3)	S2002	Sigma	25gm	6.60

APPENDIX F
CHROMATOGRAPHIC MATERIALS

Item	Stock Number	Vendor	Quantity	Price
Sepharose 4B	17-0120-01	Pharmacia LKB 800-558-1773 800 Centennial Ave. Piscataway, NJ 08854	1 liter	199.00
5'-AMP Sepharose 4B	17-0620-01	Pharmacia LKB	5gm	268.00
2', 5'-ADP Sepharose ($NADP^+$)	17-0700-01	Pharmacia LKB	5gm	268.00
DEAE-A50	17-0180-01	Pharmacia LKB	100g	120.00
Sephadex G-100, superfine	17-0061-01	Pharmacia LKB	100g	170.00
MONO Q HR 515 column	17-0546-01	Pharmacia LKB	1 each	730.00
TSK G-2000 sw HPLC Sec column	2135-260	Pharmacia LKB	1 each	1,250.00
DE-32	D8632	Sigma Chemical Company 800-325-3010 P. O. Box 14508 3500 DeCalb St. Lewis, MO 263178	50g	19.90
Q300 Anion Exchange Matrix	Q508	Synchrom 800-283-4752 P. O. Box 310 Lafayette, IN 47902-0310	5g	28.00
Propyl Hic Matrix	H504	Synchrom	5g	28.00

APPENDIX G
CHROMATOGRAPHIC EQUIPMENT

Item	Stock Number	Vendor	Quantity	Price
P-500 pumps	19-4301-01	Pharmacia LKB 800-526-3593 800 Centennial Ave. Piscataway, NJ 08854	1 each	3,880.00
V-7 Injector	19-7500-01	Pharmacia LKB	1 each	560.00
Absorbance Detector, Model 757	9000-7571	Applied Biosystems 800-831-3582 170 Williams Dr. Ramsey, NJ 07446	1 each	3,950.00
Fraction Collector	FC 203	Gilson Medical Electronics 800-445-7667 Box 27 3000 W. Beltline Hwy. Middleton, WI 53562	1 each	1,590.00
Data Acquisition System (see video & brochure, enc.)	Maxima Chromatography Workstation	Dynamic Solutions/Waters 805-658-6612 (Kersley) 2355 Portola Road, Ste. B Ventura, CA 93003	1 each	≈15,000.00
Chromaflex Columns 1.0 x 15cm	K-420870-1500	Kontes 800-255-1672 1470 Zethyr Ave. Hayward, CA 94544	1 each	87.50
Chromaflex Columns 2.5 x 60cm	K-420870-6010	Kontes	1 each	130.00

APPENDIX H
FLOW SYSTEM EQUIPMENT

Item	Stock Number	Vendor	Quantity	Price
Disposable Columns	737-1005	BioRad 800-645-3227 P.O. Box 708 220 Maple Ave. Rockville Centre, NY 11571	1 each	68.00
Teflon tubing	125-0035	BioRad	1 pack	37.00
Ismatec Peristaltic Pump	J-7618-40	Cole Parmer Instrument Co. 800-323-4340 7425 North Oak Park Ave. Chicago, IL 60648	1 each	1,796.70
Tyson tubing orange/red	J-7616-72	Cole Parmer	1 pack	16.00
Tyson tubing blue	J-7616-73	Cole Parmer	1 pack	16.00
Tyson tubing green	J-7616-74	Cole Parmer	1 pack	16.00

APPENDIX I
SUMMARY OF ENYZLUMINESCENCE PROCESSES

Enzyluminescent enzymatic reactions are the key to an ever growing family of methods used in chemical analysis. Since the recognition of an enzyme capable of the chemical reduction of the explosive TNT, coupling of this reaction with that of a sensitive and quantifiable light-producing assay has been held as a promising method for low-level explosives detection. The principle of TNT determination can be represented as follows:

1. Reduction of TNT with TNT reductase:

$$TNT + 2\,NADH + 2\,H^+ \xrightarrow{\text{TNT reductase}} TNT_{(red)} + 2\,NAD^+ + H_2O$$

2. Luminescent determination of remaining NADH:

$$NADH + H^+ + FMN \xrightarrow{\text{Flavin reductase}} NAD^+ + FMNH_2$$

$$FMNH_2 + RCHO + O_2 \xrightarrow{\text{Luciferase}} FMN + RCOOH + H_2O + light$$

All aqueous solutions were made with Milli-Q purified water that had been filtered again through a Milli-pore 0.45 µM filter and degassed 1 hour prior to use. Stock solutions were made 10 mL at a time and stored in amber glass airtight bottles at 4°C for no longer than 1 month. The purification of the enzyme TNT reductase (TNT_{ase}) has been the subject of previous reports and is detailed in this Appendix. Referring to the two-step principle of TNT determination, the stock solutions used were as follows:

- ***TNT Reductase:*** Immobilize onto activated Sepharose 4B at 0.3 unit/mL determined spectrophotometrically stored (1 g/10 mL) in 0.1 M potassium phosphate, pH 7.0, 0.2% BSA, 0.02% sodium azide, 2mM DTT, 1 mM EDTA, 10 µM FMN (dark, 4°C.).

- ***NADH:*** Stock is 7 to 10 mM determined spectrophotometrically at 340 nm (ϵ_{340} = 6220 $M^{-1}\,cm^{-1}$), stored in H_2O with 0.01 M KOH and discarded if $A_{260}/A_{340} > 2.45$ (dark, 4°C).

- ***TNT:*** Stock is 10 mg/mL = 44 mM = 44 µmol/mL = 2.2 µmol/50 µL stored in methanol in light-impermeable container at 4°C. Both glass and polypropylene eppendorf tubes have been used with no difference in apparent concentrations.

- ***Buffer:*** Two-part stock included 0.5 M KCl and 0.5 M MES throughout assay final dilutions and reactions in 100 mM KCl, 100 mM MES, both pH 7.0, filter sterilized.

- ***FMN:*** Stock is 12 mM in H_2O (dark, 4°C).

- ***n-Decanal:*** Stock is 0.15% in methanol, fresh weekly.

- ***NADH Reductase/Luciferase:*** Prepare from B. Harveyi at 4.5 units/mL and 2.2 x 107 LU/mL, respectively (stored at -20°C). Immobilize onto activated Sepharose 4B according to methods described in previous reports.

Additional stock solutions necessary were:

- ***EDTA:*** Acid form, neutralized to pH 7.0 with concentrated KOH, brought to 100 mM with H_2O.

- ***B-ME:*** Diluted directly into H_2O at 100 mM.

The final reactant solutions are referred to as the "reagents" and were made fresh daily from the stock solutions. Many variations have been explored yielding the following:

- ***NADH Reagent:*** Various levels can be used around 1 picomolar; diluted by serial dilution into buffer: 100 mM MES, pH 7.0, 100 mM KCl.

  — Buffers Na^+ and K^+-phosphate, Tris-HCl, pH 7.0 to 7.5; and TES, pH 7.0, were also tried, with MES the most favorable for both columns while contributing minimal background light.

  — KCl was introduced to increase ionic strength and facilitate more complete elution from the columns contributing to reproducibility and column longevity.

- ***FMN Reagent:*** 30 µM FMN in H_2O, 1 mM B-ME (shielded from light).

  — 10 µM to 100 µM FMN levels were used with lower levels limiting light output and higher levels contributing to pulsation and baseline degradation.

  — DTT can also be used as a reducing agent at slightly lower levels (0.25 to 0.5 mM). It seems more favorable to the luciferase reaction and contributes to column regeneration, but the resultant high background light interferes with the low-level signal.

- ***Decanal Reagent:*** 100 mM MES, pH 7.0; 100 mM KCl; 1 mM K^+-EDTA.

  — Tetra-decanal is an alternate substrate with greater light-producing capabilities, but has a much higher melting point and appears to precipitate in the presence of Na^+ (i.e., Na^+-EDTA, Na^+-NADH).

  — K^+-EDTA is not in contact with FMN until the flow cell to avoid photoreduction of FMN altering the baseline and light emission.

Liquid contact items and fittings include all flow system components from the reagent entry to final waste exit. Following the flow of the "H_2O" line this includes

- ***Reagent Reservoir:*** Small glass "Dispo columns" by BioRad holding 10 mL liquid, fitted with a polypropylene frit through which the reagent passes, luer-lock tip for easy changing and refilling. (NADH and FMN reagents must be covered with light-impermeable paper or foil.)

- ***Tubing:*** Tygon autoanalyzer tubing with inner diameter of 0.01 in. or 0.25 mm coded "orange-blue" available from Cole Parmer. This size gives a volume of only 0.5 µL/cm length or roughly 7.4 µL/15 cm, the length available for use. Coupled with the pump, this yielded a variance of flow rates of 0.002 to 0.15 mL/min/channel (single tube line). Optimum flow rate was found to be 0.04 mL/min/channel. Additional uncut Tygon was also available from Cole Parmer with the same inner diameter and was easily spliced to the pumping segments to fill out the required lengths. The third kind of tubing that proved necessary was HPLC-grade Teflon tubing.

GROWTH AND PURIFICATION OF ENTEROBACTER CLOACAE NITROREDUCTASE

Bacterial Growth and Nitroreductase Induction

Enterobacter cloacae strain 96-3 (ATCC No. 43560) is grown in the following bacteriological media: 25 mM potassium phosphate, pH 7.0; 0.2% NH_4NO_3; 0.02% $MgSO_4.7H_2O$; 1% glucose; 1% yeast extract; 0.00012% $FeSO_4.7H_2O$; 0.00012% $ZnSO_4.7H_2O$; 0.00010% $MnCl_2.4H_2O$; 0.00050% NaCl. TNT is supplied to cultures at a rate of 50 mg/L/hr. Ten liters of culture are grown for 6 hr at 30°C with shaking. The cells are harvested by centrifugation and washed with TBS. The cell yield is approximately 25 g/10 L.

Purification of *E. Cloacae* 96-3 Nitroreductase

All buffers used throughout the purification procedure contain 1 mM EDTA, 1 mM 2-mercaptoethanol, 0.02% sodium azide. Fractions collected during chromatographic steps are eluted into tubes containing an aliquot of concentrated FMN such that each fraction collected is 10 µM in FMN.

Crude Extract

- Resuspend the harvested cells in approximately five volumes of 0.1 M Tris-HCl, pH 7.5.
- Disrupt the bacteria by sonication at 4°C using a Branson Sonifier model 350 with a large tip at a power setting 60% of maximum. Perform sonication for six 30-second pulses with 1-minute intervals between each pulse.
- Remove cellular debris by centrifugation at 12,000 g for 30 minutes at 4°C.
- Decant the supernatant and save on ice.
- Discard the pellet.

Acetone Precipitation/High Speed Centrifugation

- Differentially precipitate the crude extract (previous step) using acetone as a water miscible organic precipitating agent.
- Maintain acetone (Malinckrodt) at approximately -30°C in a methanol/dry ice bath.
- Bring crude extract to 40% acetone (v/v) condition by slowly adding the acetone over a 15-minute period.
- Allow to incubate an additional 15 minute.
- Centrifuge the resulting slurry at 12,000 g for 30 minute at 4°C.
- Adjust the 40% supernatant to 70% acetone and recentrifuge as before.
- Briefly hold the pellets under a gentle stream of filtered air to drive off residual acetone associated with the pellet.
- Resuspend the moist pellets in a minimum volume 50 mM Tris-HCl, 10 M FMN, pH 7.5.
- Centrifuge at 122,000 g for 2 hours at 4°C to remove any fine particulate residue.

Q300 Preparative Anion Exchange Chromatography

- After high speed centrifugation, apply the sample to a 120-mL (2.5 x 24.5 cm) column of Q300 preparative anion exchange resin preequilibrated in 50 mM Tris-HCl, pH 7.5

- Wash the column at 3 mL/min with three column volumes of starting buffer and then develop with a 0-1 M NaCl gradient over 10 column volumes.
- Collect and analyze 7.5-mL fractions for nitroreductase activity.
- Pool the active fractions.
- Precipitate the pooled fractions by adding solid ammonium sulfate to 70% saturation.
- Centrifuge at 12,000 g for 30 minutes at 4°C.
- Resuspend the pellet in a minimal volume (approximately 15 mL) of 50 mM sodium phosphate, 20% saturated ammonium sulfate, 120 ÊM FMN, pH 7.0

Hydrophobic Interaction Chromatography

- Apply the resulting sample (from previous step) to a 10-mL (1.0 x 13.0 cm) column of Propyl HIC resin preequilibrated in 50 mM sodium phosphate, 20% saturated ammonium sulfate, pH 7.0
- Wash the column with three column volumes of starting buffer at 1 mL/min.
- Elute with a decreasing gradient of saturated ammonium sulfate from 20-0% over 12 column volumes.
- Collect and analyze 1.5-mL fractions for nitroreductase activity.
- Pool the active fractions.
- Precipitate the pooled fractions by adding solid ammonium sulfate to 70% saturation.
- Centrifuge at 12,000 g for 30 minutes at 4°C.
- Resuspend the pellet in a minimal volume of 50 mM sodium phosphate, 10 μM FMN, pH 7.0.

HPLC Size Exclusion Chromatography

- Split the resuspended pellet into 0.5-mL aliquots.
- Apply each aliquot to a GF-250 size exclusion column run in tandem with a GF-450 column (both 1.0 x 25 cm).

- Preequilibrate in 50 mM sodium phosphate, pH 7.0
- Run at a flow rate of 0.75 mL/minute.
- Collect 1.0-mL fractions.
- Pool these fractions from each run containing nitroreductase activity.

Mono Q Anion Exchange Chromatography

- Apply the combined sample to a MONO Q HR 5/5 column (0.5 x 5 cm) preequilibrated in 25 mM sodium phosphate, pH 6.0, running at 1 mL/min.
- Wash the column with 15 column volumes of starting buffer.
- Develop with a 0-350 mM NaCl salt gradient over 60 column volumes.
- Collect and analyze each 1-mL fraction for nitroreductase activity.
- Pool the active fractions.

As a final step in the purification protocol, pass the nitroreductase over the HPLC-SEC column system preequilibrated in MTEN buffer (0.025 M MES, 0.025 M Tris, 0.05 M Ethanolamine, 0.1 M NaCl, pH 7.0).

NOTE: CHROMATOGRAPHY MATERIALS AND EQUIPMENT

All chromatographic procedures described were performed at room temperature using Pharmacia, Inc. (Piscataway, New Jersey) model P-500 FPLC pumps interfaced to an IBM XT compatible laboratory computer operating with Maxima chromatography software from Dynamic Solutions, Inc. (Ventura, California). Elution profile data were collected using an Applied Biosystems (Ramsey, New Jersey) model 757 UV/Vis flow detector interfaced to the Maxima chromatography software package. Synchrom, Inc. (West Lafayette, Indiana) Q300 anion exchange and Propyl hydrophobic interaction chromatographic matrixes are packed in Chromaflex medium pressure columns from Kontes, Inc. (San Leandro, California). GF-250 and GF-450 HPLC size exclusion columns are from DuPont Co. (Wilmington, Delaware). A Mono Q HR 5/5 strong anion exchange column is purchased from Pharmacia.

GROWTH AND PURIFICATION OF B. HARVEYI OXIDOREDUCTASE AND LUCIFERASE

Bacterial Growth

Vibrio harveyi strain 392 are grown in complete media consisting of 0.3% glycerol, 3% NaCl, 0.01% MgSO4, 0.7% $Na_2HPO.4H_2O$, 0.1% KH_2PO4, 0.05% $(NH_4)_2PO_4$, 0.5% tryptone, 0.3% yeast extract. Growth is initiated with a 1% inoculum and grown at 25°C with aeration 3/4 log phase.

Purification of *B. Harveyi* Oxidoreductase and Luciferase

Crude Extract

- Thaw frozen cells (300-400 g) overnight in the cold (4°C) and lyse the following morning in cold 10^{-2} M EDTA, 10^{-4} M DTT, and 0.001 M K_3PO_4, pH 7.0, at a ratio of 1 g cells/8 mL lysis buffer.
- Stir the lysate slowly in the cold for 1 to 2 hours in a large Erlenmeyer flask.
- Monitor and maintain the lysate at pH 7.0 with 0.1 M KOH.
- Remove the cell debris by centrifugation at 13,000 g in a Sorvall RC5B refrigerated centrifuge for 30 minutes at 0 – 4°C.
- Decant the supernatant carefully into a large beaker and keep on ice.

Adsorption on DE-32

- Allow Whatman DE-32 cellulose (in the phosphate form) stored in distilled water to settle.
- Decant the supernatant.
- Add the moist DE-32 directly to the crude lysate in a ratio of about 150 mL of DE-32 to 1 L of crude lysate.
- Stir the mixture gently, maintaining the pH at 6.7 – 7.0 at 4°C.
- After 30 minutes, withdraw a sample of 1 mL, and remove the DE-32 by centrifugation in a microcentrifuge.

- Assay the supernatant for luciferase and both oxidoreductase activities. If more than 10-15% of the initial activity is present, add a little more DE-32, stirring and sampling until only 10-15% of the initial activities are present.

Elution of Enzymes from DE-32

- Remove the DE-32 from the crude extract by filtration through Miracloth (Calbiochem) on a large Buchner funnel.

NOTE: The base of a nalgene pipet jar makes a good form for tracing out and cutting a circle of Miracloth to fit the large plastic Buchner funnel. Trace and cut the Miracloth when it is wet. If you do it when it is dry, it changes shape as it gets moist.

- Pour the crude lysate containing the suspended DE-32 through the 5 x 50 cm buchner funnel under vacuum.

- Rinse the DE-32 with a few hundred mL of cold 0.05 M K_3PO_4 with 5 x 10^{-4} M DTT, pH 7.0.

- Resuspended the DE-32 in a minimum volume of the same buffer, and gently pour into a 5 x 50 cm column (plugged with Miracloth on the bottom).

- Allow to drain off any excess 0.05 M K_3PO_4. **Do not let it run dry.**

- When the level of the buffer has reached the bed of the DE-32, start a linear gradient of K_3PO_4 buffer containing 5 x 10^{-4} M DTT from 0.05 to 0.5 M at pH 7.0 (1600 mL of each).

- Collect fractions of 10 mL overnight at 4°C. This procedure separates the protein from the bulk of the nucleic acids in the crude extract.

Concentration by Ammonium Sulfate

- Assay the fractions for luciferase and oxidoreductase activity the next morning.

- Pool together the fractions with the oxidoreductase activities. Separately pool the fractions with the luciferase activity.

NOTE: If you do not get a good separation, consider luciferase to be secondary when pooling fractions.

- Precipitate the oxidoreductase with ammonium sulfate between 35 and 75% saturation on ice.

- Precipitate luciferase between 50 and 75% saturation on ice.

- Centrifuge the precipitate at 10,000 g using a GSA rotor at 4°C.

- Resuspend the precipitates in minimum volume of 0.2 M K_3PO_4, 0.5 mM DTT, pH 7.0.

- Dialyze against the same buffer 3 times.

- Suspend the precipitate into the 0.2 M K_3PO_4 buffer gently.

- Freeze the dialyzed luciferase at -20°C for later purification.

Chromatography of Sephadex A50

- Apply the dialyzed oxidoreductase to a 3.8 x 70 cm column of DEAE-A40 sephadex.

- If highly purified oxidoreductase is needed, equilibrate the column with 0.2 M K_3PO_4 with 5 x 10^{-4} M DTT, pH 7.0, and elute with the same buffer.

- Using these conditions, separate the oxidoreductase with the NADH:FMN oxidoreductase emerging first. Yields are comparatively low and time required for chromatography is about 2 to 3 days.

- If a higher yield is desired, with a lower quality enzyme preparation, equilibrate the column with 0.35 M K_3PO_4, pH 7.0, with 5 x 10^{-4} M DTT and elute with the same buffer. Using these conditions, the oxidoreductase is separated essentially by molecular weight with the NADPH:FMN oxidoreductase eluting first, which takes about 24 hours.

- Collect 10-mL fractions at 4°C.

- If luciferase is present, remove it by elution with 0.35 M K_3PO_4 and 5 x 10^{-4} M DTT, pH 7.0, in 3 to 4 days.

Concentration with Ammonium Sulfate

- Precipitate the oxidoreductase and any luciferase separately after elution from the DEAE-A50 column.

- Pool the fractions containing the activity.
- Precipitate the oxidoreductase between 35 to 75% saturation of ammonium sulfate in the cold.
- Precipitate the luciferase between 40 and 75% saturation.
- Collect the precipitate by centrifugation at 10,000 g.
- Gently resuspend in a minimum of cold 0.1 M K_3PO_4 with 5 x 10^{-4} M DTT.
- Dialyze against the same buffer three times.
- Remove the enzymes and store frozen at -20°C until needed.

Luciferase Purification

- Purify with precipitated and dialyzed luciferase from the DE-32 column to 90 to 95% homogeneity by chromatography on DEAE-A40 equilibrated with 0.35 M K_3PO_4 with 10-4 M DTT, pH 7.0
- Elute the column with the same buffer, and pool the active fractions, precipitated with ammonium sulfate between 40 and 75% saturation and dialyzed vs. 0.1 M K_3PO_4 with 10^{-4} M DTT, pH 7.0, 3 times.
- Store the preparation frozen.
- If a highly purified preparation is desired, the enzyme is taken through the following steps.

Chromatography on Sephadex G-100

- Apply the enzymes separately to a Sephadex G-100 fine column, 2.3 x 90 cm, equilibrated with 0.1 M K_3PO_4 and 5 x 10^{-4} M DTT, pH 7.0, in a volume of less then 5.0 ml.
- Elute with the same buffer at 4 drops/min at 4°C.
- Collect 40-drop fractions.
- Pool the active fractions, about 40 to 50 mL.

- Concentrate the enzyme in an Amicon Ultrafiltration cell using a PM 10 membrane at 50 to 55 psi N_2 down to 5 to 10 mL final volume at 4°C.

- After releasing the pressure, swirl the cell by hand gently 1 minute to release any protein on the surface of the membrane.

Affinity Chromatography on 5-ft AMP Column

- Apply the PM 10 concentrated NADH:FMN oxidoreductase to the 5-ft AMP affinity column (about 40 µmol NADH/min).

- Elute with 24 mL of the following NAD solutions in 0.05 M K_3PO_4 with 5×10^{-4} M DTT, pH 7.0:

  0 M
  5×10^{-4} M
  1×10^{-3} M
  5×10^{-3} M
  1×10^{-2} M

- Collect 3-ml fractions.

- Pool the active fractions.

- Use 80 mL of 10^{-2} M NAD in 0.05 M K_3PO_4 and 5×10^{-4} M DTT, pH 7.0, as the stock solution.

- Use all the 10^{-2} NAD in the final elution step.

- Wash the column with 1 M NaCl, followed by equilibration with 0.05 M K_3PO_4 with 5×10^{-4} M DTT, pH 7.0.

- Keep the column in a cold box. Eluted volume is about 35 to 40 mL.

Affinity Chromatography on NADP Agarose

- Apply the PM 10 concentrated NADPH:FMN oxidoreductase to the NADP agarose affinity column (about 40 µmol NADPH/min).

- Elute with 25 mL of the following concentrations: 0, 5×10^{-6}, 10^{-5}, 5×10^{-5}, and 10^{-4}.

- Collect 3-mL fractions.
- Pool the active fractions.
- A good stock volume is 80 mL of 10^{-4} NADP in 0.05 M K_3PO_4, 5 x 10^{-4} M DTT, pH 7.0.
- Use all the 10^{-4} M NADP in the final elution step.
- Wash the column with 1 M NaCl, equilibrated with starting buffer.
- Keep the column in a cold box; eluted volume is about 35 to 40 mL.

Concentration of Purified Enzymes by Amicon Ultrafiltration and Aquacide

- Concentrate the highly purified enzymes from the affinity columns in an Amicon ultrafiltration cell using a PM 10 filter at 50 to 55 psi N_2 down to a final volume of 5 to 10 mL.
- Further reduce this volume to 1 to 2 mL by placing the enzyme ion in a dialysis bag and covering it with dry Aquacide II in the cold.
- When the volume has been reduced to the desired level, remove the enzyme.
- Place the enzyme in a fresh dialysis bag and dialyze against 0.1 M K_3PO_4, pH 7.0. The NADH enzyme needs DTT to remain fully active; the NADP enzyme does not.
- Freeze the enzymes or store them in the cold at 4°C in the presence of 0.02% azide.
- Remove any contaminating oxidoreductase from the luciferase by passing the luciferase through both the affinity columns.
- Wash the columns with NAD or NADP followed by 1 M NaCl and reequilibrate with 0.05 M K_3PO_4 and DTT after the luciferase has been eluted. Luciferase does not stick to the affinity columns.

WASHING GELS USED IN BACTERIAL ENZYME PURIFICATION

DE-52

- Pour the spent resin out of the column and into a large beaker.
- Add 2 L of 0.5 N KOH.
- Stir the resin gently a few times over 30 minutes at room temperature.
- Filter the resin through miracloth on a large buchner funnel.
- Wash with 2 L of 0.4 N KOH followed by deionized water to remove KOH.
- Place the resin back into the large beaker.
- Add 2 L of 0.5 H_3PO_4 and stir gently a few times for 30 minutes.
- Filter the resin again through miracloth followed by deionized water to remove the acid.
- Wash the resin with 2 L of 0.1 M K_3PO_4, pH 7.0, followed by 2 L distilled water.
- Resuspend the cleaned resin in distilled water and store in the cold.
- If the resin is not to be used for some time, add 0.02% sodium azide.
- Do not let resin dry or become less than saturated with liquid at any time during washing or storage.

DEAE-A50

- Pour the spent gel out of the column into a large Buchner funnel containing a miracloth filter.
- Wash the gel with deionized water at room temperature until swollen, followed by 4 L of 0.5 N KOH.
- Wash the gel again with deionized water until the KOH is removed and the gel is swollen.
- Wash with 4 L of 0.5 N H_3PO_4, followed by deionized water.

- Wash the gel finally with 2 L 0.35 M K_3PO4, pH 7.0, and store in the same.
- If the gel is not to be used for some time, add 0.02% sodium azide.
- Do not allow the gel to become dry or even less than saturated with liquid.
- De-gas under vacuum before another use.

Sephadex G-100

- Wash this gel in the column by running 0.1 M K_3PO_4, pH 7.0, through it in the cold for 12 to 24 hours.

IMMOBILIZATION OF ENZYMES ON SEPHAROSE

Activation of Sepharose CL-4B

- Wash 10 g of wet gel with 500 mL of cold deionized water.
- Suspend the washed gel in 20 mL of cold 2 M K_2CO_3.
- Prepare a solution of 1 g CNBr in 1 mL of acetonitrile.
- Add the CNBr solution to the gel slurry while stirring on ice.
- An initial flocculation should be noted, which clears after approximately 2 minutes.
- After 5 minutes, wash the CNBr activated gel with 600 mL of cold water followed by 500 mL of cold 100 mM pyrophosphate buffer, pH 8.0.

Immobilization of TNT_{ase}

- Prepare a solution of 1 mg purified TNT_{ase} (dialyzed against 100 mM pyrophosphate); 1 mL 100 mM pyrophosphate, pH 8.0; and 12 mg bovine serum albumin.
- Add 1 g of activated Sepharose 4B to this solution and incubate at 4°C overnight with mixing.
- After incubation, wash the suspension off with 200 mL of cold 100 mM phosphate, 100 mM DTT, pH 7.0; 500 mL of cold 100 mM phosphate, 1 M NaCl, 100 mM DTT, pH 7.0; and 300 mL of first solution prepared.

- After the wash cycle, suspend the gel in 10 mL of 100 mM phosphate, 2.0 mM DTT, 1.0 mM EDTA, 0.02% NaN_3, and 0.2% BSA.

SOLUBLE LUCIFERASE, OXIDOREDUCTASE, AND NITROREDUCTASE ASSAYS

Luciferase (Benckea harveyi)

Solutions

- For decanal stock: Add 5 µL of decanal to 10 mL of distilled water and shake vigorously. This turbid solution is stable all day on ice.

- For decanal buffer: Add 100 µL of decanal stock solution to 9.9 mL of 0.1 M K_3PO_4 buffer, pH 7.0. This solution is stable for about 3 to 4 hours on ice.

- For FMN-EDTA: Prepare a solution of 1.5×10^{-4} M in FMN and 5×10^{-4} M in EDTA in 0.1 M K_3PO_4 buffer, pH 7.0. Adjust to a final pH of 7.0. Deoxygenate this solution by flushing with N_2 gas for 15 to 20 minutes. Store solution in the dark at 4°C. It is stable for about 1 month.

Procedure

- Add 0.6 mL decanal buffer solution to as many assay tubes as needed and allow to equilibrate to room temperature.

- Take up 100 µL of FMN-EDTA in a syringe and place under a Tungsten light source for photoreduction, about 30 seconds.

- Add 0 to 100 ÊL of luciferase to the assay tube and mix gently. Place the tube in the photometer.

- Inject the $FMNH_2$ in the syringe into the assay tube through the gasket in the photometer with the high voltage and recorder on.

NADH:FMN Oxidoreductase

Stock Solutions

- K_3PO_4-Na pyrophosphate buffer, pH 8.5.

- 1.3×10^{-2} M FMN in distilled water. Store in cold and dark. This is stable for about 1 month.

- 2.0 x 10^{-3} NADH in 0.1 M K_3PO_4 buffer, pH 7.0. Keep on ice. This should be good all day.

Procedure

- Add 0.88 mL of pyrophosphate buffer to a cell followed by 10 µL of FMN and 10 µL of enzyme.
- Add 100 µL of NADH last, mix gently, and place in the spectrophotometer.
- Measure the reaction at 340 nm vs. a buffer-FMN blank.
- If more than 10 µL of enzyme is assayed, lower the volume of pyrophosphate buffer accordingly to maintain a constant volume of 1.0 mL final.

$$\frac{\mu\text{mol NADH oxidized/min}}{\text{mL enzyme}} = \frac{(\Delta\,\text{OD/s})\,(60\text{s/min})\,(0.001\text{ L/mL})\,(10^6\,\mu\text{mol/mol})}{(6200)\,(0.01\text{ mL enzyme})}$$

NADPH:FMN Oxidoreductase

Stock Solutions

- K_3PO_4 buffer, pH 5.6
- 1.3 x 10^{-2} M FMN in distilled water.
- 2.0 x 10^{-3} M NADPH in 0.1 M K_3PO_4 buffer, pH 7.0.

Procedure. Same as for NADH:FMN oxidoreductase.

Coupled Luciferase-Oxidoreductase Assay

Stock Solutions

- Decanal stock: Add 5 µL of decanal to 10 mL of distilled water.
- Decanal buffer: Add 100 µL of decanal stock to 9.9 mL of 0.1 M K_3PO_4 buffer, pH 7.0.
- FMN: 1.5 x 10^{-4} M in 0.1 M K_3PO_4, pH 7.0.
- NADH or NADPH: 2.0 x 10^{-3} M in 0.1 M K_3PO_4 buffer, pH 7.0.

Procedure

- Add 0.5 mL of decanal buffer to as many assay tubes as are needed and allow to equilibrate to room temperature.

- Add 10 μL of 1.5×10^{-4} M FMN to an assay tube, followed by 0 to 100 μL of luciferase and 0 to 100 μL of either oxidoreductase, and mix the tube gently.

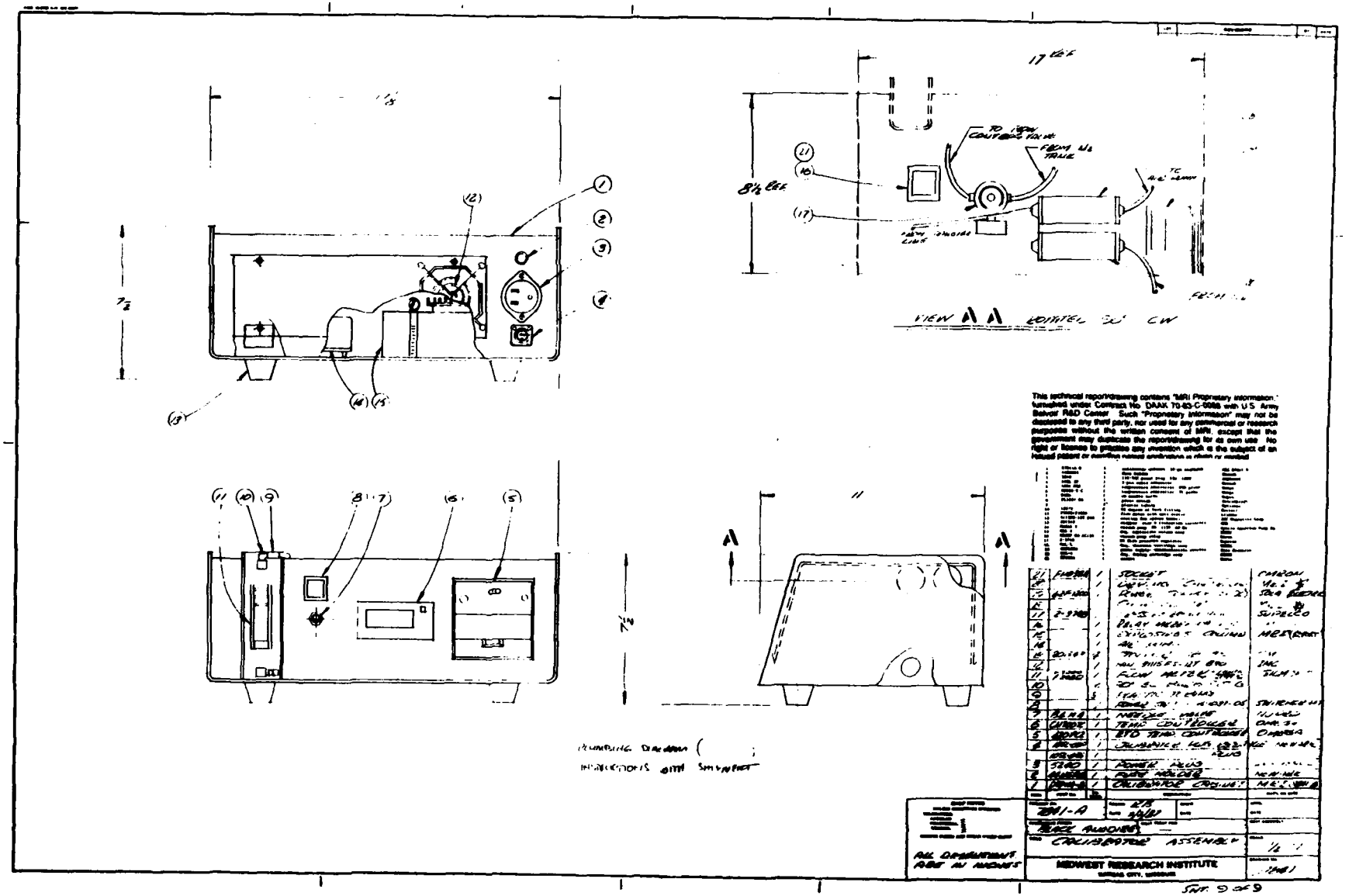
- Add 10 μL of NADH or NADPH to the assay tube, mix gently, and quickly place into the photometer. The light output is proportional to the concentration of substrates or to the product of the concentration of the luciferase and oxidoreductase if the substrates are saturating.

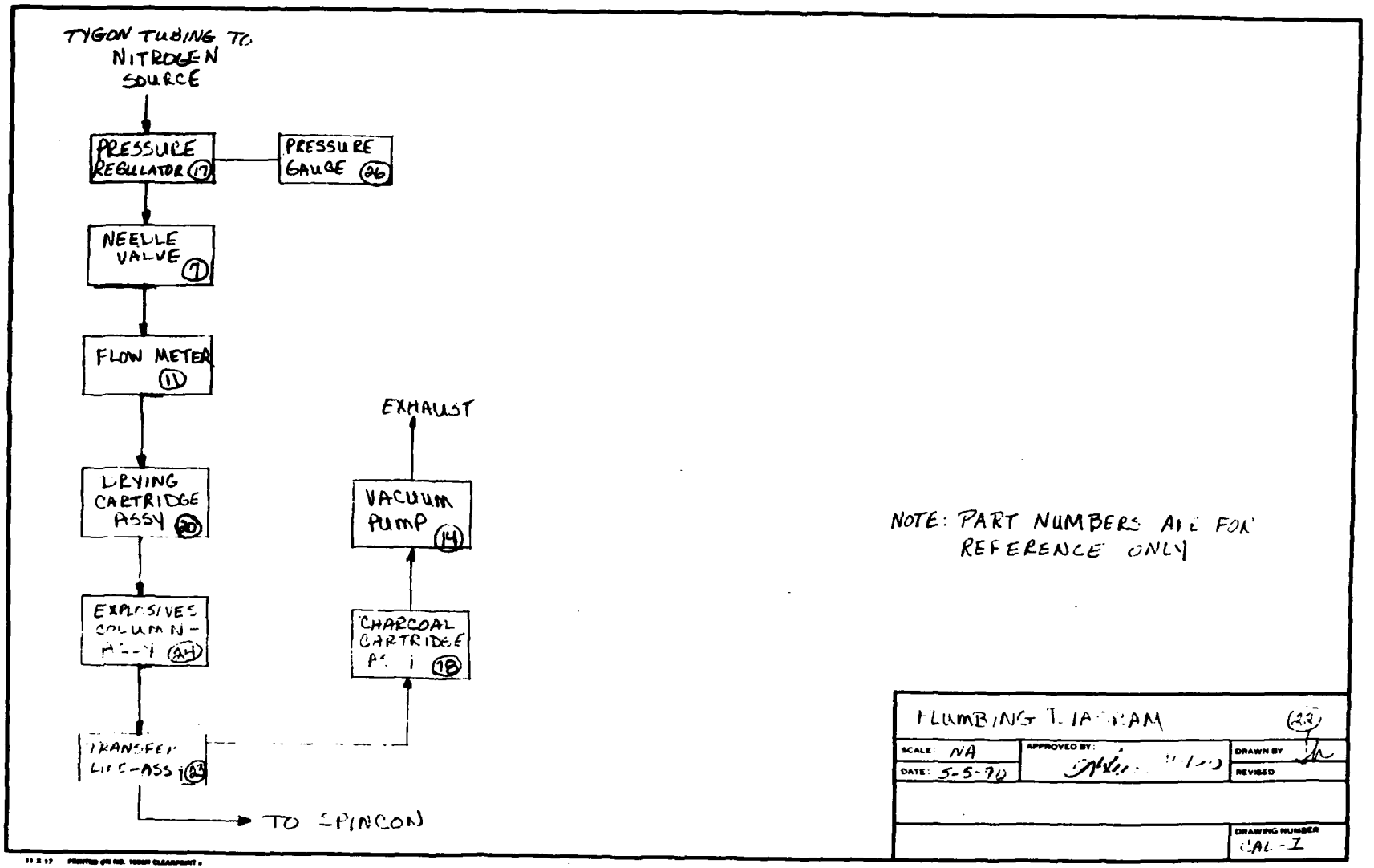
Nitroreductase Assay

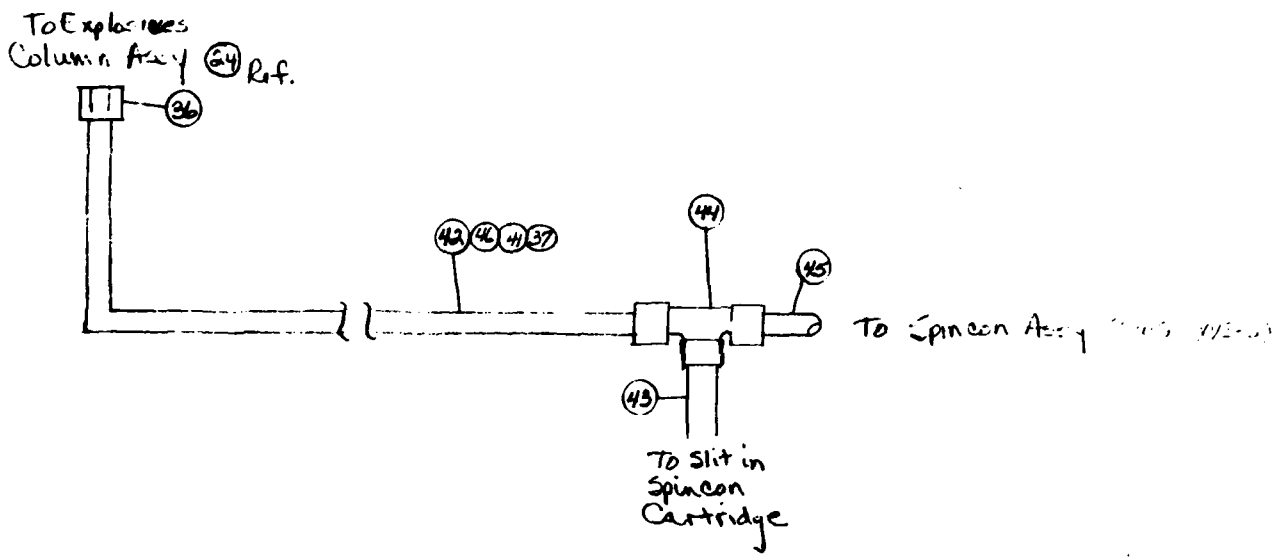
- Assay nitroreductase activity by measuring the initial rate of NADH oxidation and subsequent decrease in absorbance at 340 nm. One unit of activity is defined as 1 μmole of NADH oxidized/min at 25°C.

- A standard assay is developed for use during the purification procedure. The assay mixture contains 45 μM TNT, 0.30 mM NADH in a final volume of 1.0 mL of a buffer composed of 50 mM acetate, 50 mM MES, 100 mM Tris, pH 7.0.

APPENDIX J
SAMPLE TRAIN DRAWINGS




TYGON TUBING TO NITROGEN SOURCE
PRESSURE REGULATOR (17)
PRESSURE GAUGE (26)
NEEDLE VALVE (7)
FLOW METER (11)
DRYING CARTRIDGE ASSY (20)
EXPLOSIVES COLUMN - ASSY (24)
TRANSFER LINE-ASSY (23)
TO SPINCON
EXHAUST
VACUUM PUMP (14)
CHARCOAL CARTRIDGE ASSY (18)
NOTE: PART NUMBERS ARE FOR REFERENCE ONLY
PLUMBING DIAGRAM (22)
SCALE: NA
DATE: 5-5-90
APPROVED BY:
DRAWN BY
REVISED
DRAWING NUMBER
CAL-I



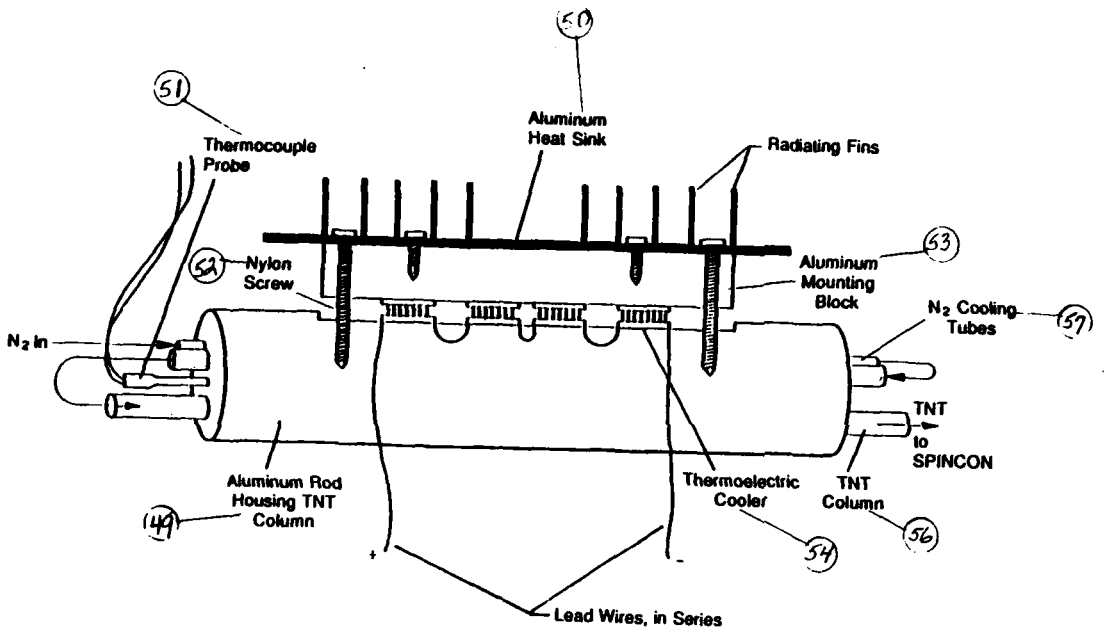
NOTES:
1. Fiberglass cloth (46) is wrapped over assy for insulation and is held with copper wire (47)
2. Thermocouple (41) follows along length of glass tube from tee to union
3. Strip heater (37) follows along length of glass tube (42) from tee (36) to union (43)
4. 3/16 OD glass tube is bent to interface with Spincon cartridge when Calibrator is placed atop XVS module

ITEM	PN	QTY	DESCRIPTION	MFR
36	T-400-6	1	1/4 Teflon union	Swagelok
37		1	strip heater	Omega
41		1	thermocouple	Omega
42		1	3/16 OD, 1/16 ID glass tube	Optional
43		1	1/4 OD glass tube, 2" long	Optional
44	T-400-3	1	1/4 Teflon union tee	Swagelok
45		1	1/4 OD Tygon tubing	Optional
46		As req'd	fiberglass cloth	Optional
47		As req'd	copper wire	Optional

TRANSFER LINE ASSY (23)

SCALE: NA
DATE: 5-5-93
APPROVED BY:
DRAWN BY
REVISED

DRAWING NUMBER CAL-2

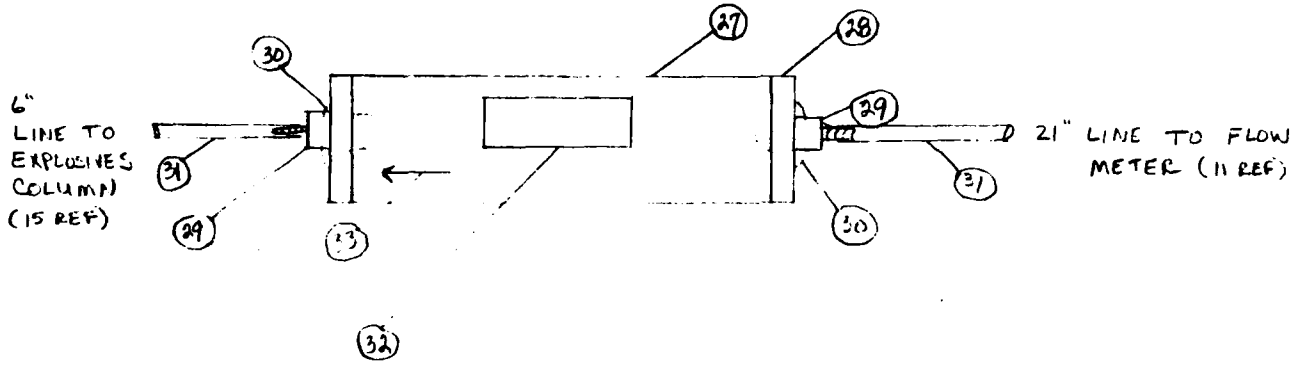


ITEM	PIN	AMT	DESCRIPTION	MFG
49	CAL-8	1	dwg, explosives column housing fab	BRDEC
50		1	finned aluminum heat sink	Optional
51		1	TC probe	Optional
52		4	nylon screws	Optional
53		1	aluminum mounting block	Optional
54		4	thermoelectric coolers	Optional
55		as req'd	0.5" foam insulation	Optional
56			1/4" glass TNT column	Optional
57			1/4" glass nitrogen tubes	Optio al

EXPLOSIVES COLUMN ASSY

SCALE:	APPROVED BY:	DRAWN BY
DATE: 5-5-89		REVISED

DRAWING NUMBER CAL-3

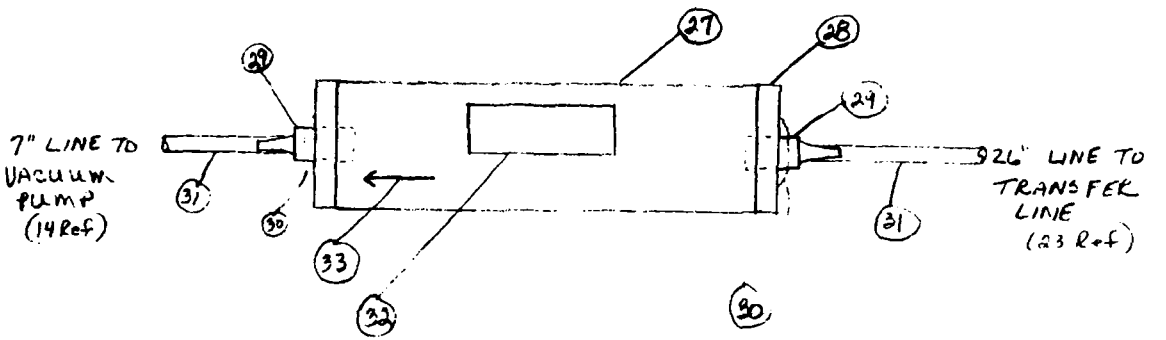


NOTE: CARTRIDGE IS FILLED WITH SILICA GEL (38), MOLECULAR SIEVE (39), AND CHARCOAL (34). EXIT END IS FILLED WITH (35)

ITEM	AN	AMT	DESCRIPTION	MFG
27		1	polycarbonate cylinder, 6" long, 1" dia	Optional
28		2	polycarbonate discs, 1/4" thick, 1" dia	Optional
29		2	1/4 MNPT to barb brass fittings	Optional
30		as reqd	epoxy bead	Optional
31	R3603	27"	1/4 OD Tygon tubing	Optional
32		1	1" x 2" Velcro pad	Optional
33		1	red arrow showing direction of flow	Optional
34		as reqd	charcoal	Optional
35		as reqd	glass wool	Optional
38		as reqd	silica gel (calcium sulfate)	Optional
39		as reqd	molecular sieve	Optional

DRYING CARTRIDGE ASSY (37)

SCALE: NA
DATE: 5-11-90
APPROVED BY:
DRAWN BY
REVISED
DRAWING NUMBER: DA-4



NOTE: CARTRIDGE IS FILLED WITH CHARCOAL (34) + THE ENTRANCE END OF THE CARTRIDGE IS FILLED WITH (35)

ITEM	PN	AMT	DESCRIPTION	MFG
27		1	polycarbonate cylinder, 4" long, 1" dia	Optional
28		2	polycarbonate discs, 1/4" thick, 1" dia	Optional
29		2	1/4 MNPT to barb brass fittings	Optional
30		As reqd	epoxy bead	Optional
31	R3603	33"	1/4 OD Tygon tubing	Optional
32		1	1" x 2" Velcro pad	Optional
33		1	red arrow showing direction of flow	Optional
34		As reqd	charcoal	Optional
35		As reqd	glass wool	Optional

CHARCOAL CARTRIDGE ASSY.

SCALE: NA

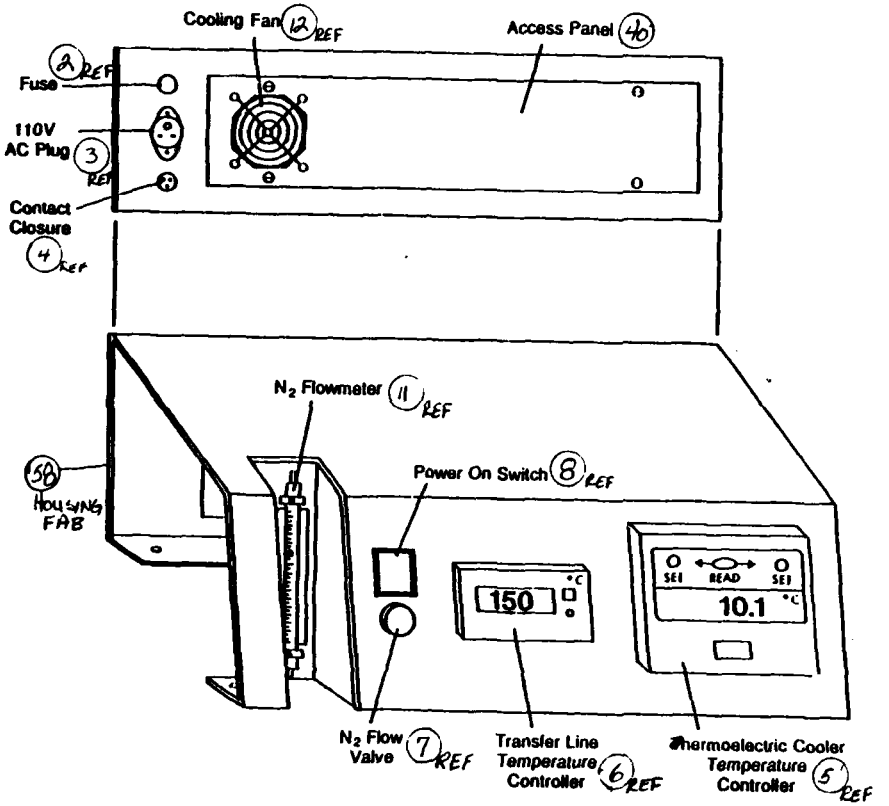
DATE: 5-11-90

APPROVED BY: [illegible]

DRAWN BY: JL

REVISED

DRAWING NUMBER: CA-5

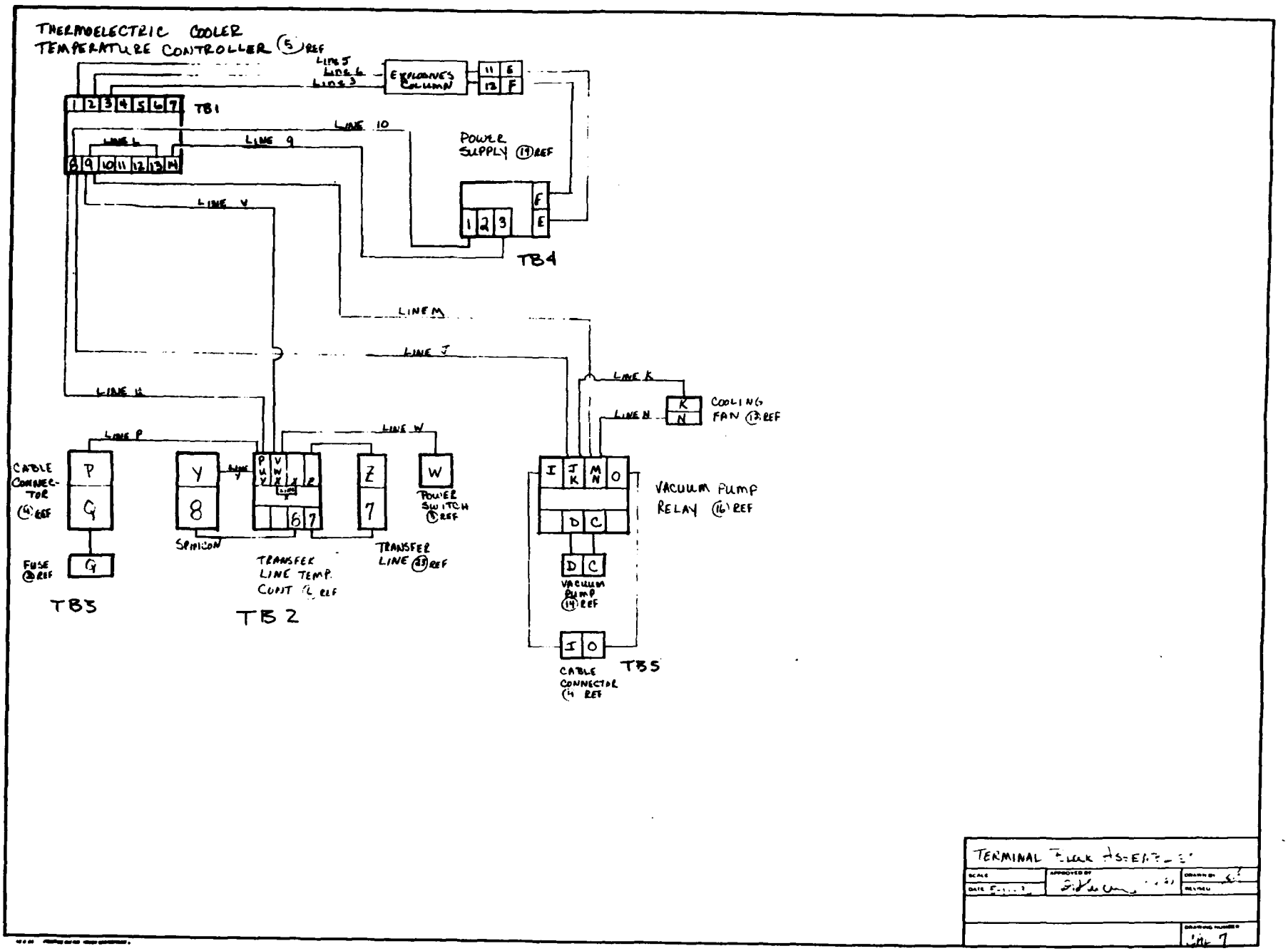


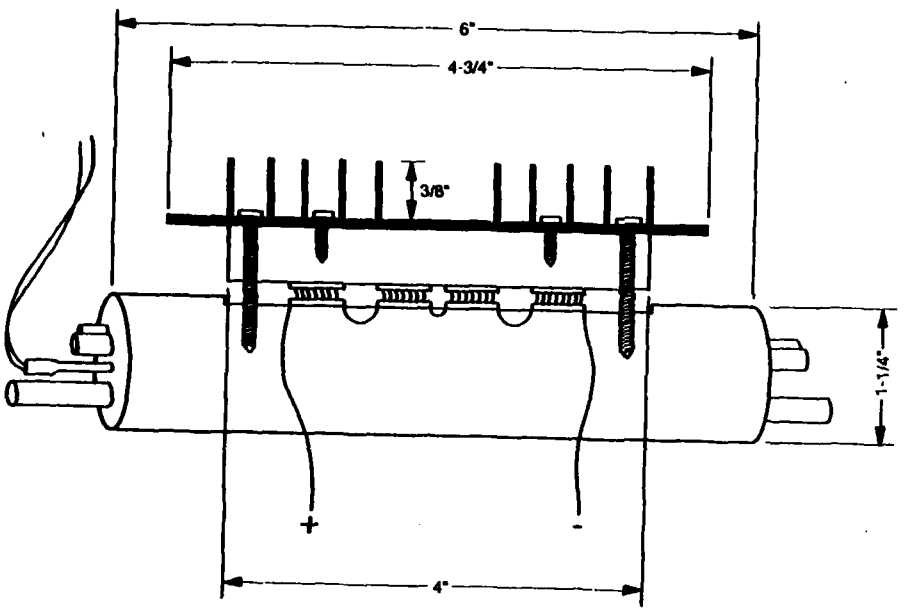
ITEM	PN	AMT	DESCRIPTION	MFG
40	CAL-y	1	access panel	BRDEC
58		1	dwg. front panel housing fab	BRDEC

FRONT PANEL ASSY (45)

SCALE: NA	APPROVED BY:	DRAWN BY
DATE: 11-11-90		REVISED

DRAWING NUMBER CAL-6

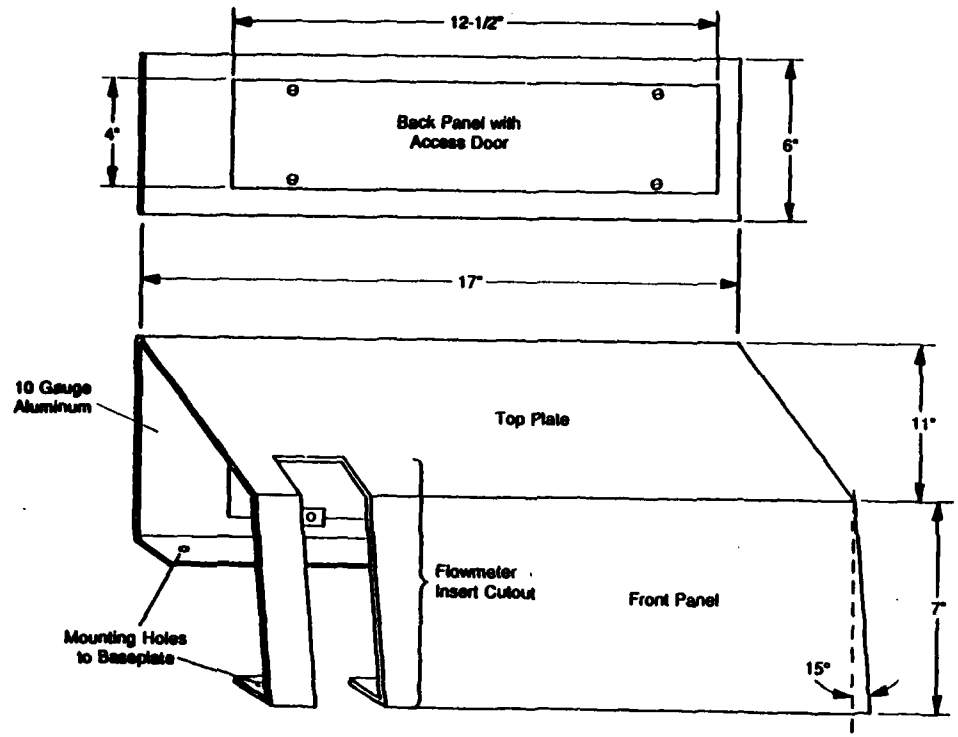

THERMOELECTRIC COOLER
TEMPERATURE CONTROLLER (5) REF
LINE J
LINE L
LINE 3
EXPANSION COLUMN
11
12
E
F
1 2 3 4 5 6 7
TB1
LINE 10
LINE L
LINE 9
8 9 10 11 12 13 14
POWER SUPPLY (19) REF
F
E
1 2 3
TB4
LINE V
LINE M
LINE J
LINE K
LINE H
K
N
COOLING FAN (13) REF
LINE N
LINE P
LINE W
CABLE CONNECTOR (4) REF
P
Q
Y
8
LINE Y
P U V
V W X
1 2
LINE Z
Z
7
W
POWER SWITCH (5) REF
I J K M N O
D C
VACUUM PUMP RELAY (16) REF
FUSE (2) REF
Q
SPINICON
TRANSFER LINE TEMP. CONT (1) REF
6 7
TRANSFER LINE (23) REF
TB3
TB2
D C
VACUUM PUMP (14) REF
I O
CABLE CONNECTOR (14) REF
TB5
TERMINAL BLOCK ASSEMBLY
SCALE
DATE
APPROVED BY
DRAWN BY
REVISED
DRAWING NUMBER
UNIT 7



NOTE. ALUMINUM AND ALLOY OPTIONAL.

GLASS TUBES = 1/4" OD
1/8" ID

EXPLOSIVES COLUMN HOUSING FAB (49)		
SCALE: NA	APPROVED BY:	DRAWN BY:
DATE: 11-11-90	11/14/90	REVISED
		DRAWING NUMBER: CAL-B

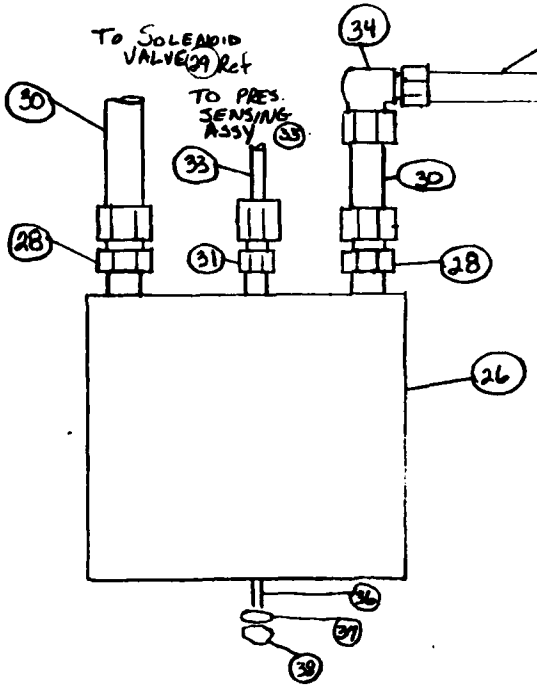


NOTE: PAINT BLACK OR BLACK ANODIZE

FRONT PANEL HOUSING FAB

SCALE: NA	APPROVED BY:	DRAWN BY:	
DATE: 11-11-90		REVISED	
		DRAWING NUMBER	CAL-9

APPENDIX K
VAPOR GENERATOR DRAWINGS

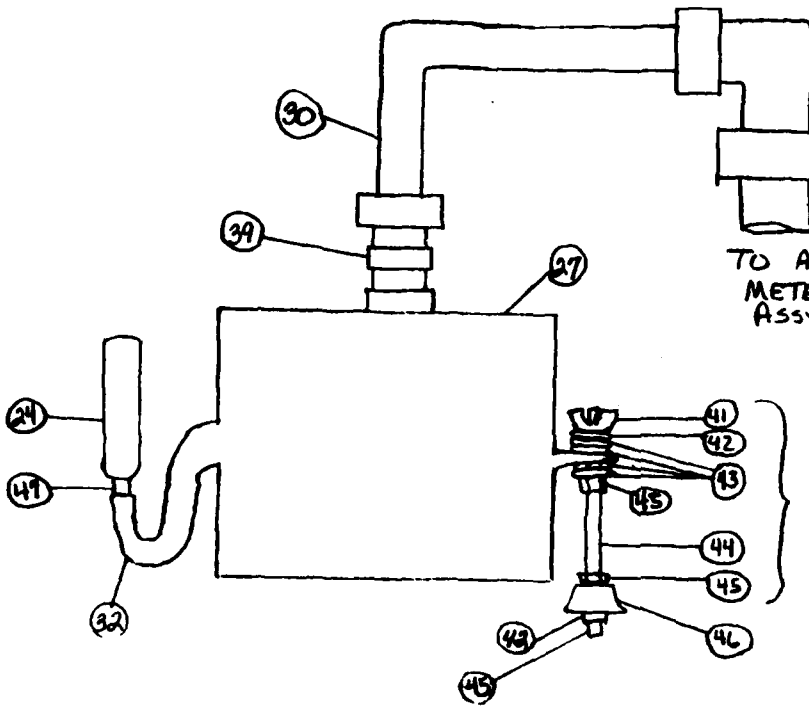


ITEM	P/N	QUANT	DESCRIPTION	MFG
26		1	500 ml water trap	Optional
28	SS-810-1-8	2	1/2 SS tube to MNPT connector	Supplied
30		15"	3/4 OD aluminum tubing formed as reqd	Optional
30		14"	3/4 OD aluminum tubing formed as reqd	Optional
30		2"	3/4 OD aluminum tubing formed as reqd	Optional
31	SS-220-1-2	1	1/8 SS tube to MNPT connector	Supplied
33		12.5"	1/8 SS tubing (pressure sensing line)	Optional
34	SS-1210-9	1	3/4 SS union elbow	Supplied
35	XVS-3	1	dbg, pressure sensing assy	MSDSC
36		1	screw, upstand to base of water trap	Optional
37		1	lock washer	Optional
38		1	nut	Optional

WATER TRAP ASSY (25)

SCALE: NA
DATE: 5-20-90
APPROVED BY: [signature] 5/7/90
DRAWN BY: [signature]
REVISED

DRAWING NUMBER: XVS-1

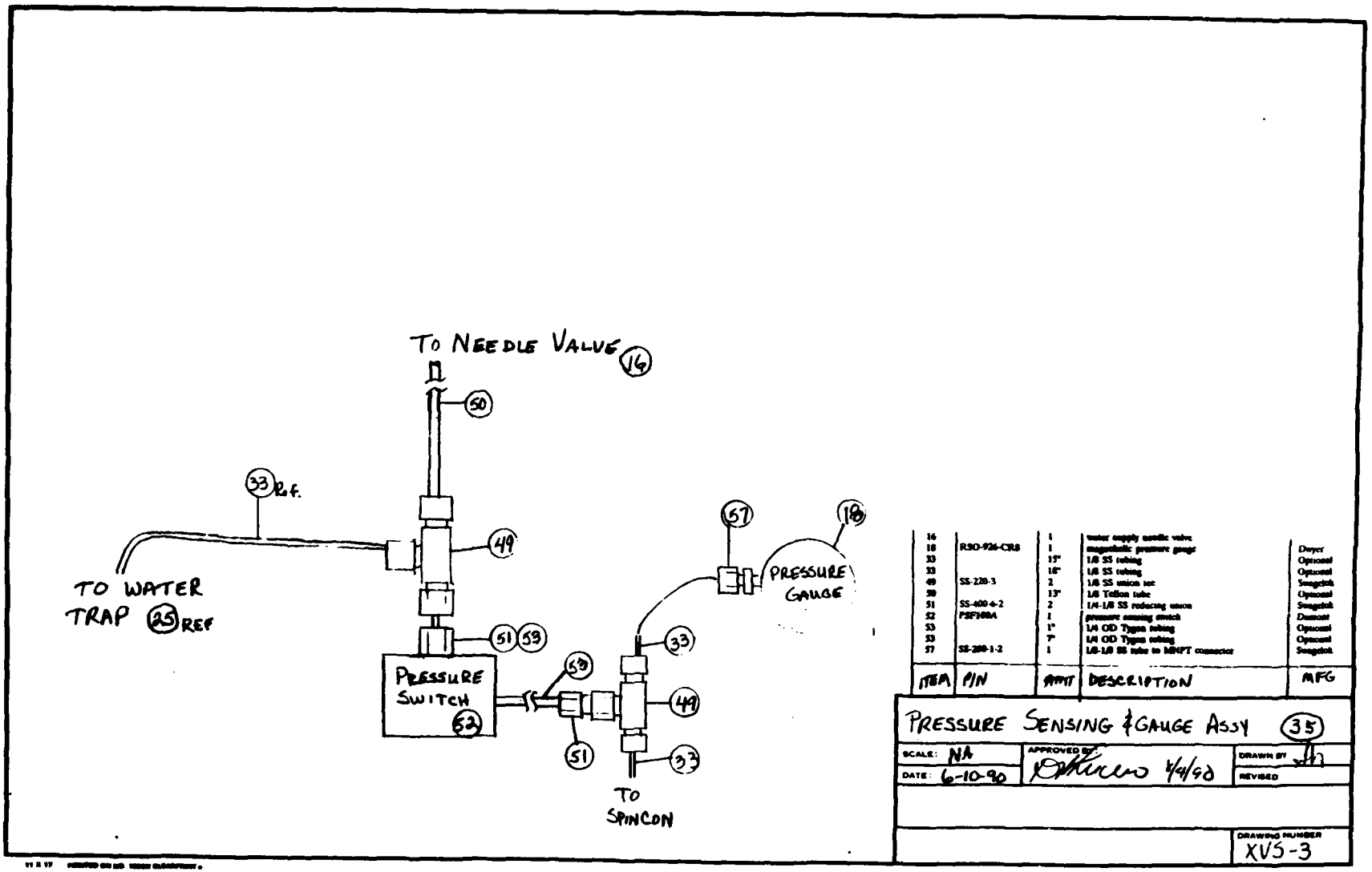


ITEM	PIN	AMT	DESCRIPTION	MFG
24		1	muffler	Optional
27		1	high volume blower	Unknown
29	XVS-5	1	mtg, air flow meter assy	BRDEC
38		2.5'	3/4 OD aluminum tubing	Optional
39		13'	3/4 OD aluminum tubing formed as req'd	Optional
32	SS-16-RSE-12	1	1" to 3/4 reducing street elbow	Swagelok
30	SS-1210-1-12	1	3/4 tube to MNPT connector, brass	Swagelok
40	SS-1210-9	1	3/4 union elbow	Swagelok
41		3	wing nut	Optional
42		6	metal washer	Optional
43		12	rubber washer	Optional
44		3	3/8 screws, 7.5" long	Optional
45		9	3/8 nuts	Optional
46		3	vibration isolators	Optional
47		1	1" pipe coupling	Unknown

HIGH VOLUME BLOWER ASSY (23)

SCALE: NA
DATE: 6-10-90
APPROVED BY: [signature] 5/7/90
DRAWN BY: [signature]
REVISED

DRAWING NUMBER XVS-2



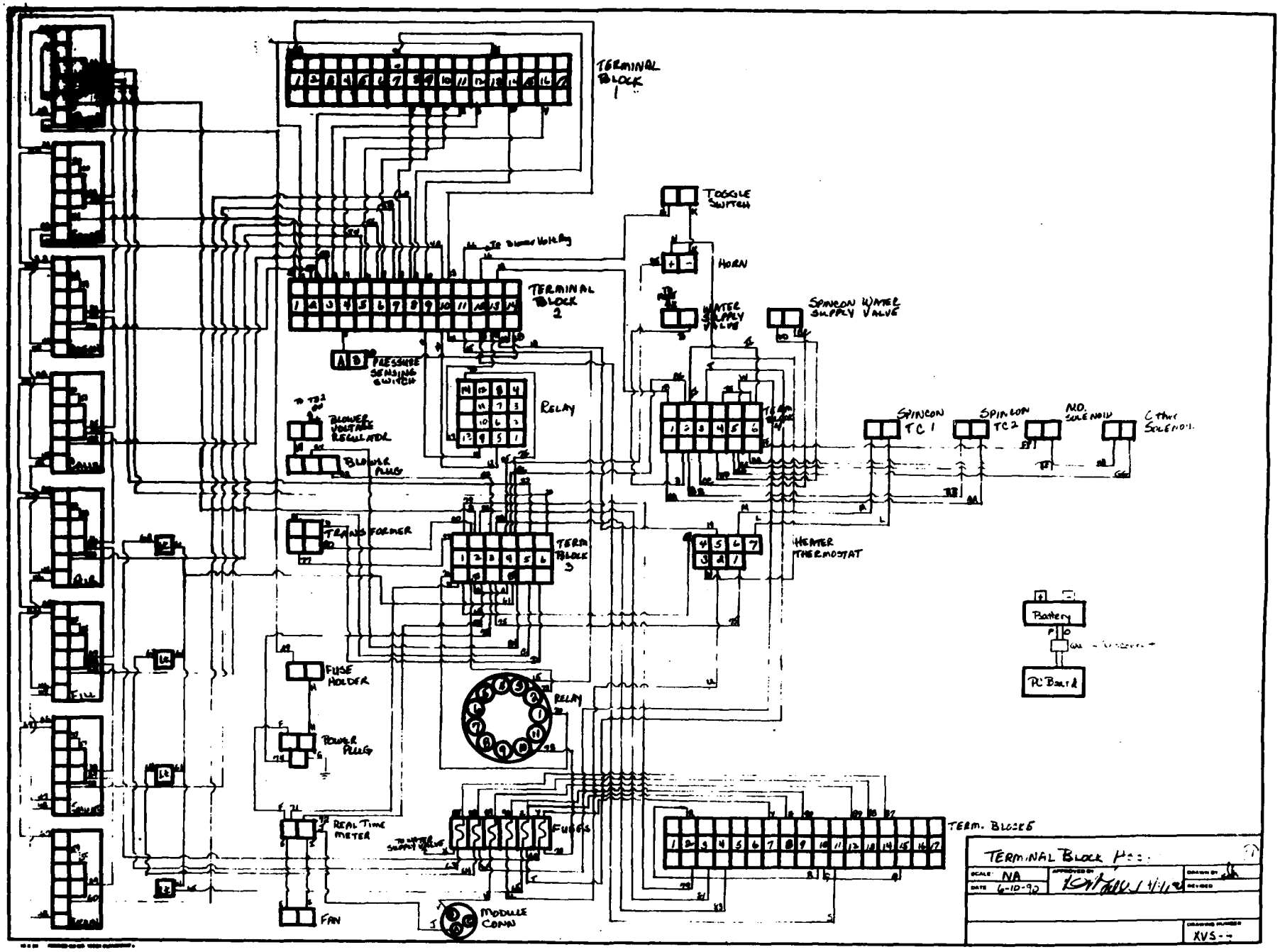
ITEM	P/N	QNTY	DESCRIPTION	MFG
16		1	water supply needle valve	
18	R30-926-CR8	1	magnehelic pressure gauge	Dwyer
33		15"	1/8 SS tubing	Optional
33		18"	1/8 SS tubing	Optional
49	SS-220-3	2	1/8 SS union tee	Swagelok
20		13"	1/8 Teflon tube	Optional
51	SS-400-6-2	2	1/4-1/8 SS reducing union	Swagelok
52	PSF108A	1	pressure sensing switch	Dumont
53		1'	1/4 OD Tygon tubing	Optional
33		7'	1/4 OD Tygon tubing	Optional
57	SS-200-1-2	1	1/8-1/8 SS tube to MNPT connector	Swagelok

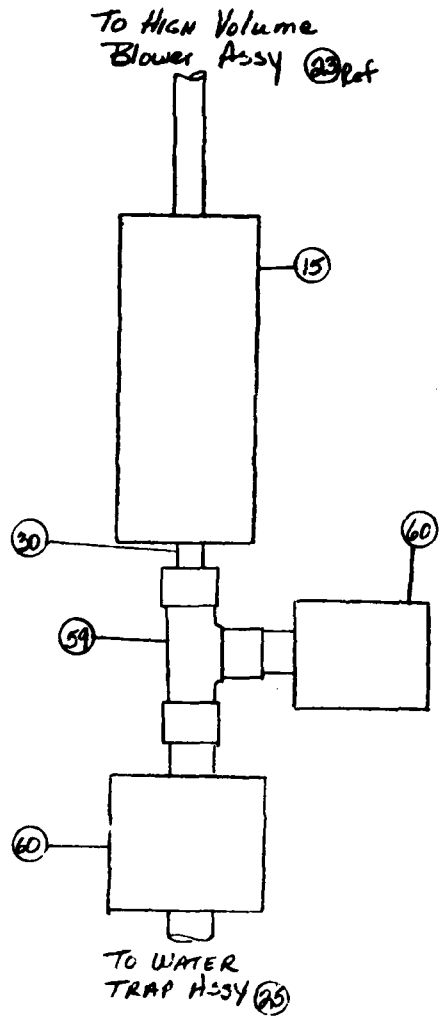
PRESSURE SENSING & GAUGE ASSY (35)

SCALE: NA | APPROVED BY: [signature] 4/4/90 | DRAWN BY: [illegible]

DATE: 6-10-90 | REVISED

DRAWING NUMBER XVS-3


TERMINAL BLOCK 1
TERMINAL BLOCK 2
TOGGLE SWITCH
HORN
WATER SUPPLY VALVE
SPINCON WATER SUPPLY VALVE
PRESSURE SENSING SWITCH
RELAY
BLOWER VOLTAGE REGULATOR
BLOWER PLUG
SPINCON TC1
SPINCON TC2
N.O. SOLENOID
OTHER SOLENOID
TRANSFORMER
TERM BLOCK 3
HEATER THERMOSTAT
Battery
PC Board
FUSE HOLDER
POWER PLUG
RELAY
REAL TIME METER
FUSES
TERM. BLOCK 6
FAN
MODULE CONN
TERMINAL BLOCK Hook-up
SCALE: NA
DATE: 6-10-92
DRAWN BY
REVISED
DRAWING NUMBER
XVS--

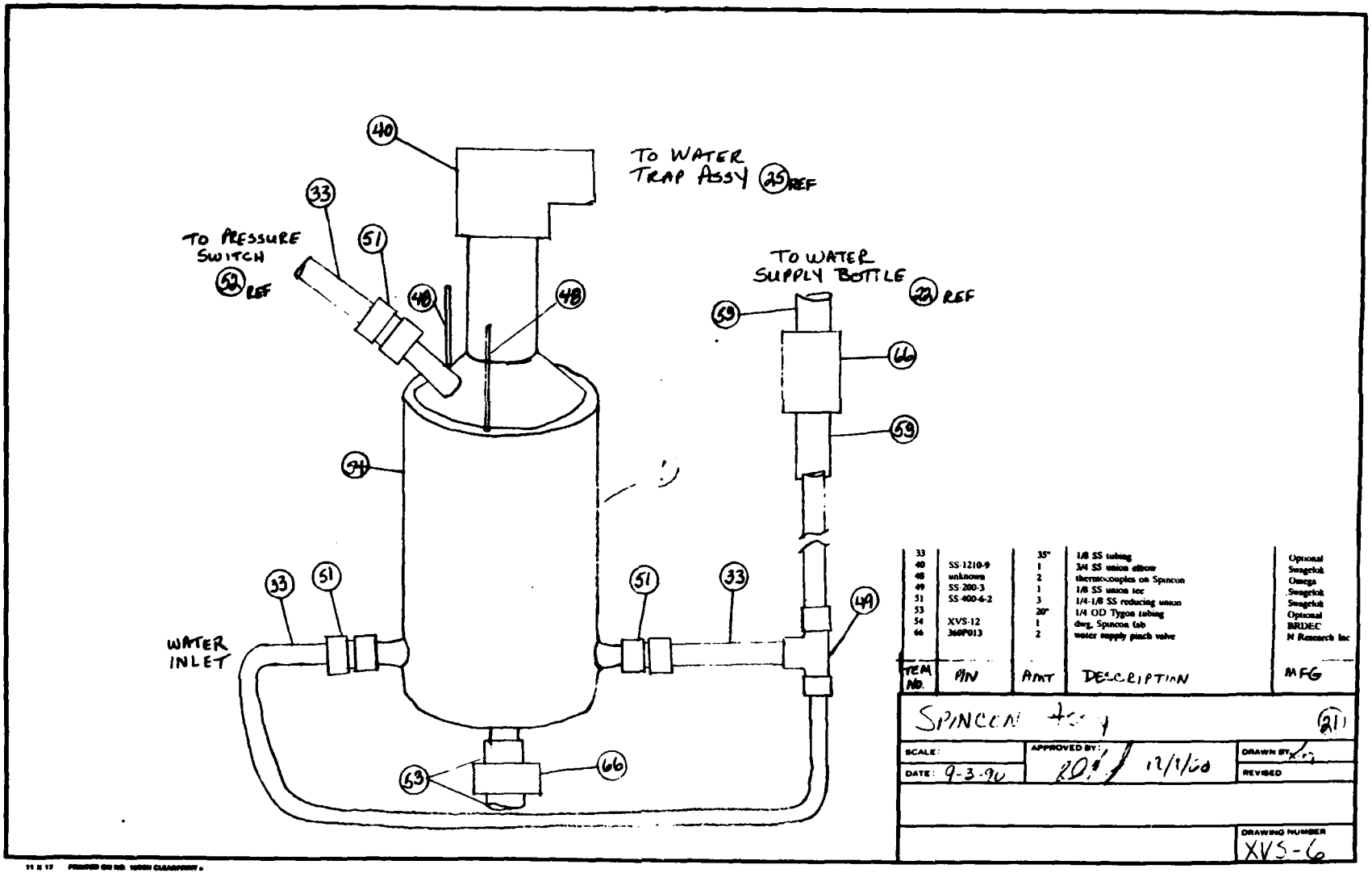


ITEM NO.	P/N	QTY	DESCRIPTION	MFG
15	RMC-106-SSV	1	high volume air flow meter	Dwyer
25	XVS-1	1	drop, water trap assy	BRIDEC
30		1.5"	3/4 OD aluminum tubing	Optional
59	B-1210-3	1	3/4 OD brass union tee	Swagelok
60	8210C34	2	solenoid valves for air above	Asco

HIR FLOW METER Assy (29)

SCALE: NA
DATE: 4-3-90
APPROVED BY: [signature] 12/7/90
DRAWN BY: [illegible]
REVISED

DRAWING NUMBER: XVS-5



ITEM NO.	P/N	QNT	DESCRIPTION	MFG
33		35"	1/8 SS tubing	Optional
40	SS-1210-9	1	3/4 SS union elbow	Swagelok
48	unknown	2	thermocouples on Spincon	Omega
49	SS 200-3	1	1/8 SS union tee	Swagelok
51	SS 400-6-2	3	1/4-1/8 SS reducing union	Swagelok
53		20"	1/4 OD Tygon tubing	Optional
54	XVS-12	1	dwg, Spincon fab	BRDEC
66	360P013	2	water supply pinch valve	N Research Inc

SPINCON ASSY (21)

SCALE:

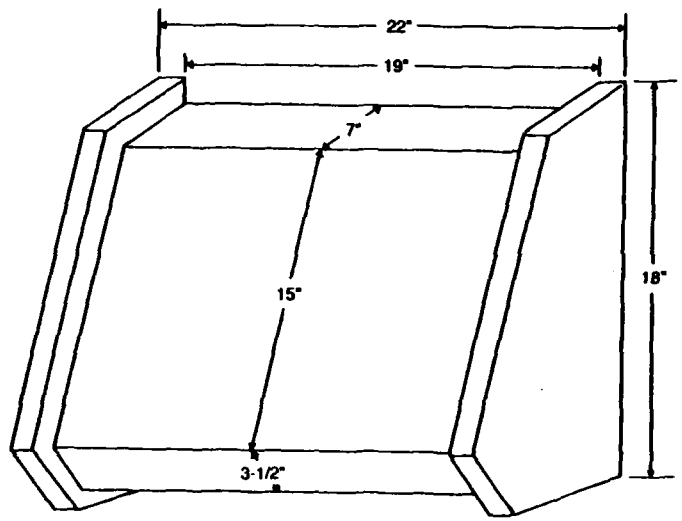
DATE: 9-3-96

APPROVED BY: 12/1/96

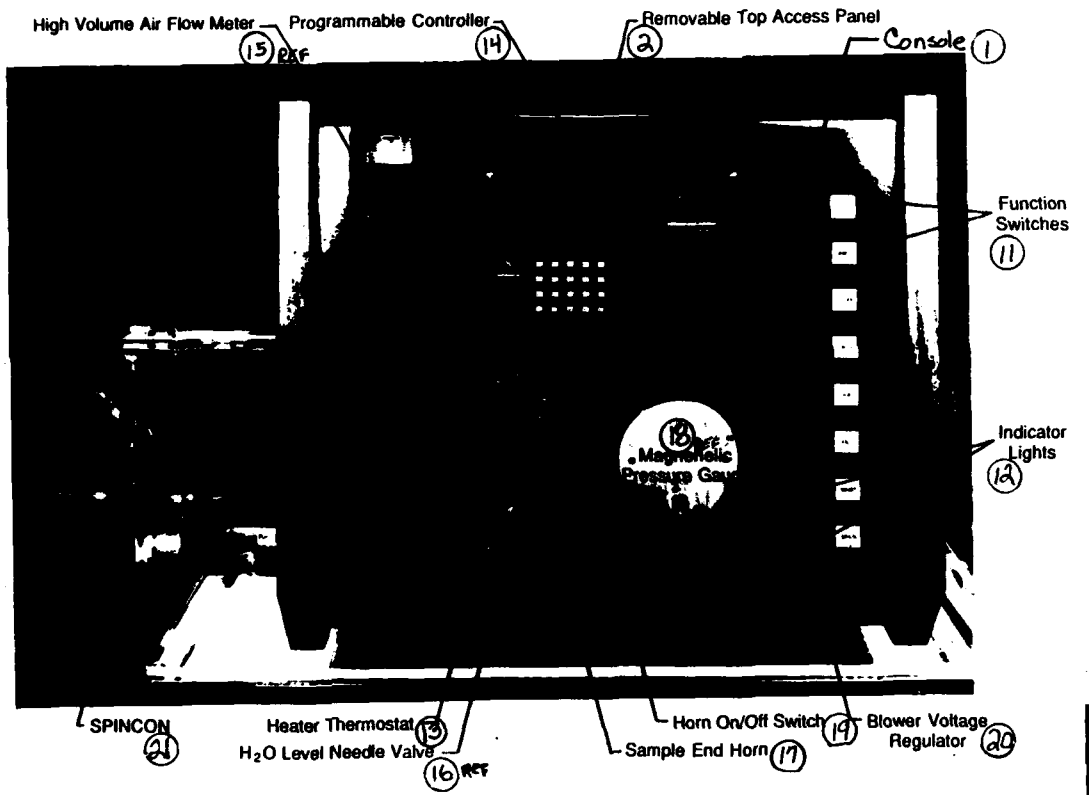
DRAWN BY:

REVISED

DRAWING NUMBER XVS-6



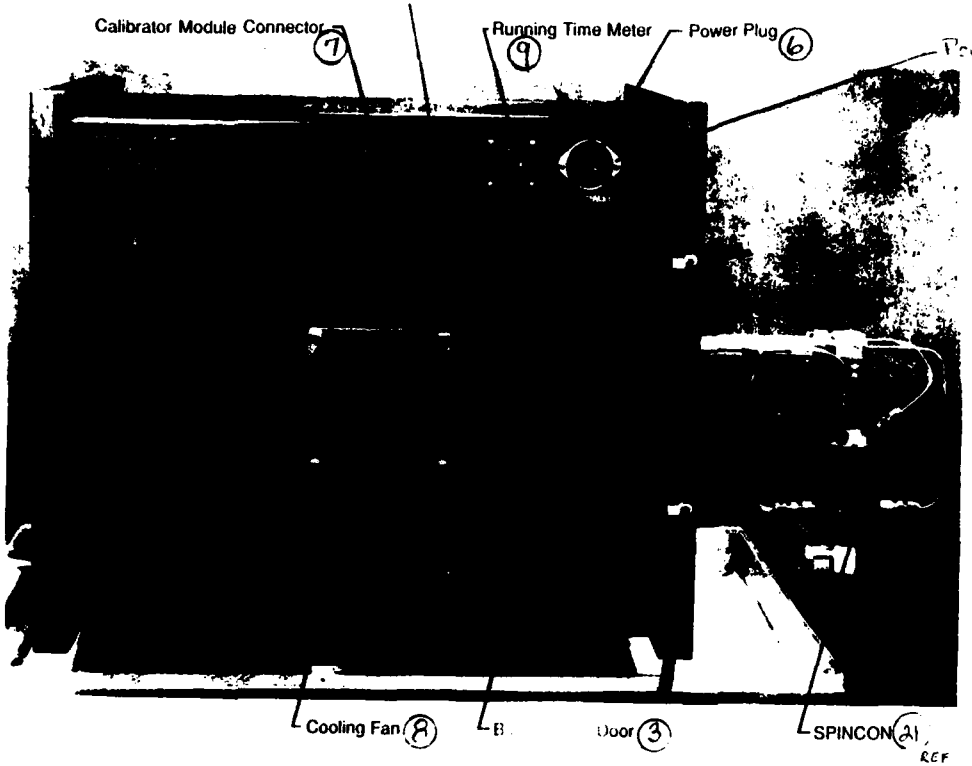
XVS CONSOLE - FAB			(1)
SCALE: NA	APPROVED BY: [signature]	12/1/90	DRAWN BY [signature]
DATE: 9-24-90			REVISED
Material: 10 ga aluminum			DRAWING NUMBER XVS-7



ITEM NO	QTY	AMT	DESCRIPTION	MFG
1	XVS-7	1	dwg, XVS cabinet fab	BRDHC
2		1	top access panel, 16 x 8 in	MRI
11		8	function switches: power, sample, air, calibrate, clean, fill, shunt, drain	Optional
12		4	indicator lights for switches	Optional
13	6100	1	Spincon heater thermostat	Omega
14	unknown	1	Series One programmable controller	GE
17	70	1	sample end horn (temperature alarm)	Omega
19		1	horn on/off switch	Optional
20	PCA 1050	1	Ohmstrol blower voltage regulator	Ohmstr Mfg
21	XVS-6	1	dwg, Spincon assy	BRDHC

FRONT PANEL, ASSY (62)

SCALE: NA	APPROVED BY: DJ 12-24-90	DRAWN BY: JL
DATE: 7-24-90		REVISED
		DRAWING NUMBER: XVS-8



ITEM	P/N	HIT	DESCRIPTION	
3		1	back access door, 18.5 x 13 .38 in	MRI
4		1	fuse holder	Optional
5	unknown	1	fuse, 15A	Buss
6	unknown	1	10 ft power cord with power plug	Amphenol
7	108E-3P	1	three pin cable connector	Unknown
8	WS21071F1110	1	cooling fan (super biser)	IMC Magnetics
9	7010 010	1	elapsed time meter	EIC
10		1	power plug receptacle	Optional

Fuse holder kit

SCALE: N.A.	APPROVED BY:	DRAWN BY:
DATE: 1-24-9		REVISED
		DRAWING NUMBER

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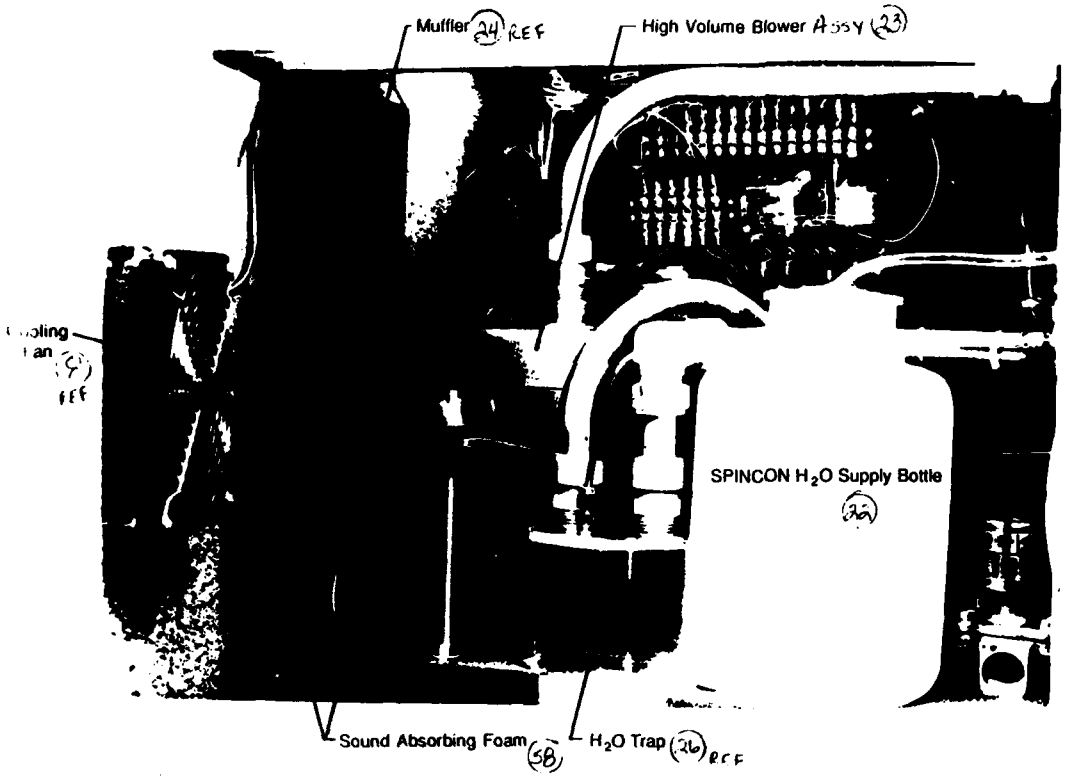

22	TS 614	1	2000 ml water supply bottle	Kartell
23	XVS 2	1	dwg. high volume blower assy	BRDEC
58		as reqd	0.5" foam insulation	Optional

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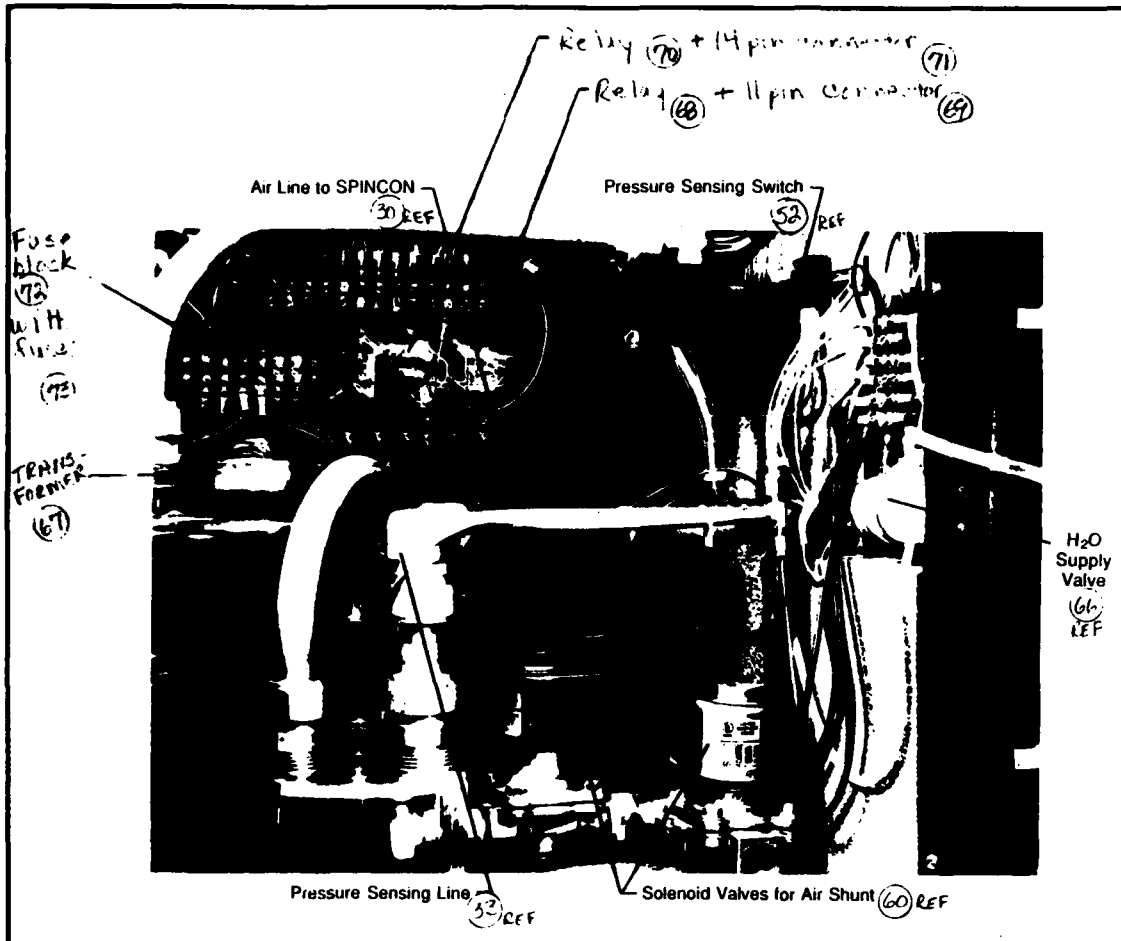

67	23V253	1	transformer	Unknown
68	MY4 US	1	relay	Omron
69		1	11 pin connector for relay	Optional
70	MKSUP UA/AC20	1	relay	Omron
71		1	14 pin connector for relay	Optional
72	3532	2	3 fuse block	Unknown
73		6	fuses, 2 amp, 250 V	Optional

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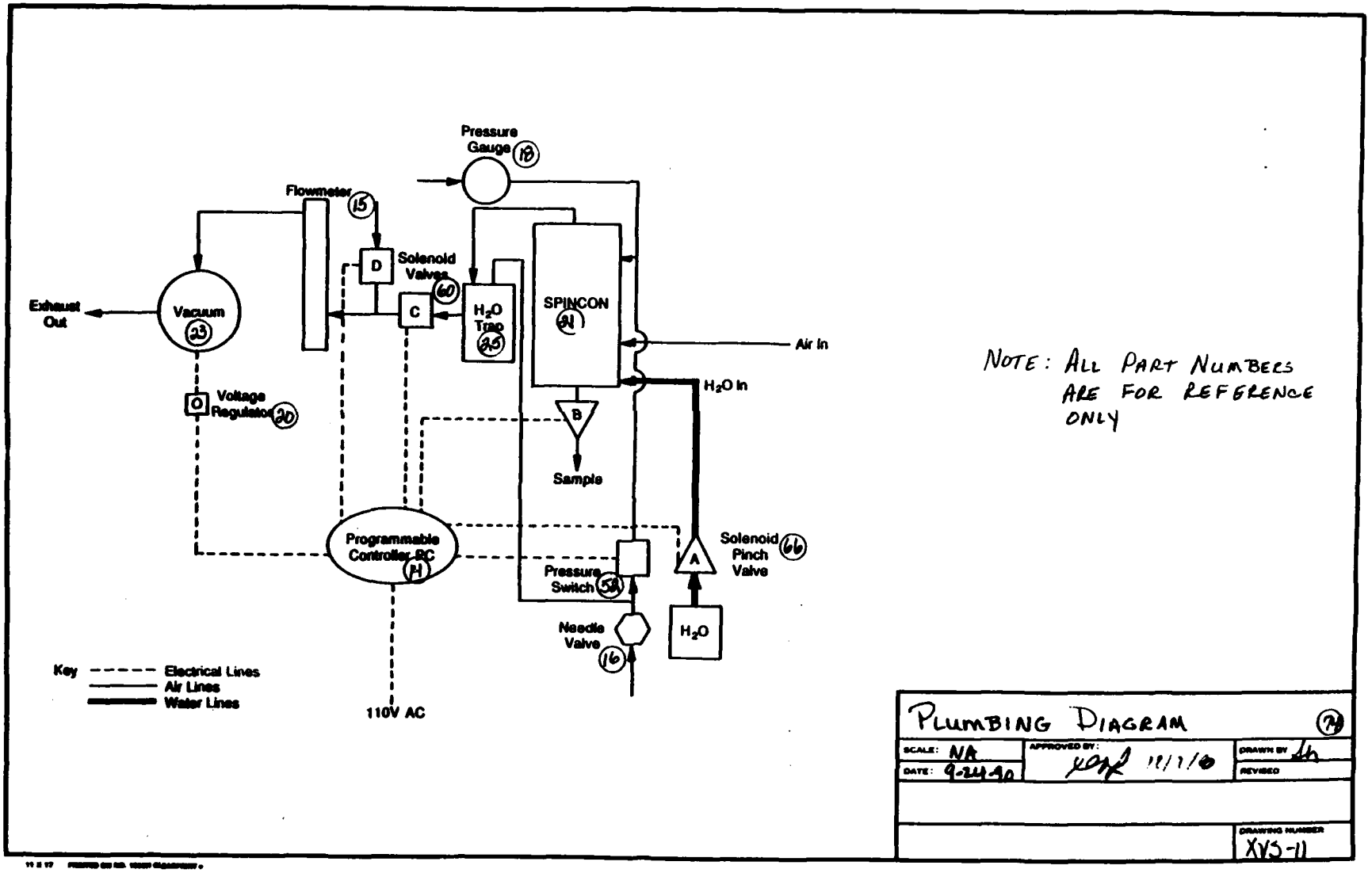

NOTE: ALL PART NUMBERS ARE FOR REFERENCE ONLY

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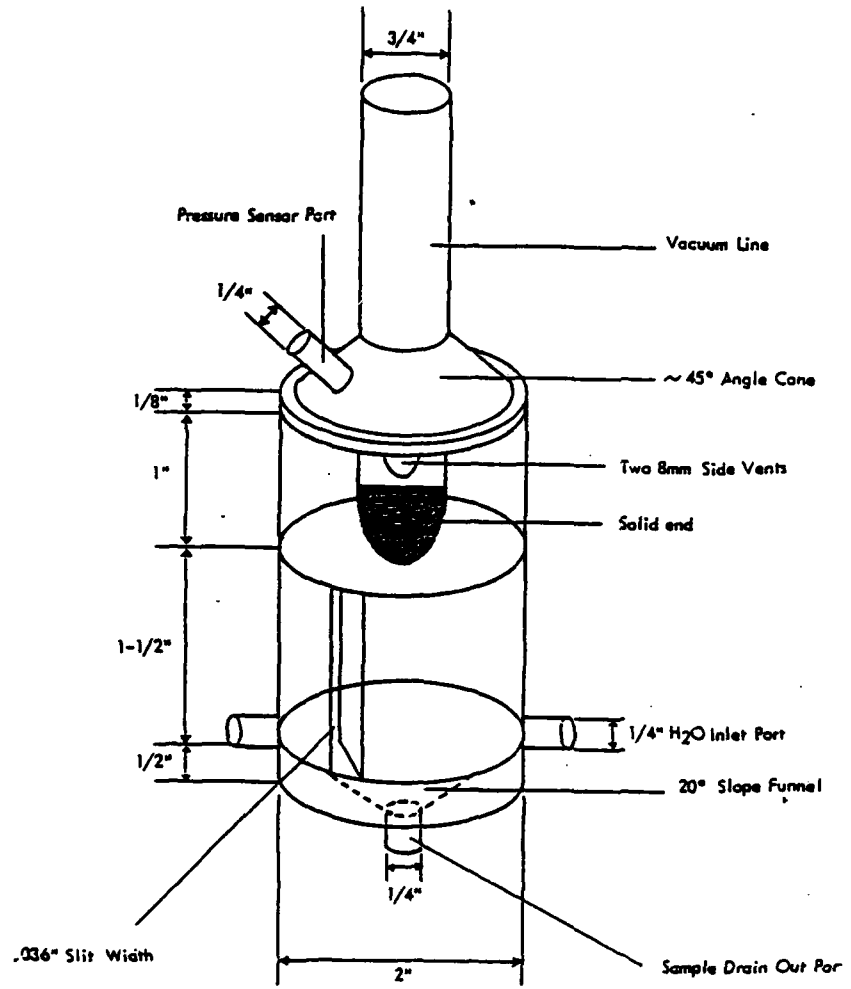

NOTE: ALUMINUM

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